



Is immunotherapy with fungal vaccines effective?

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Purpose of review

Although allergen immunotherapy (AIT) for fungi has been performed for many years, evidence clearly demonstrating its clinical benefit are still lacking. Here, we reviewed the available studies assessing efficacy and safety of AIT for molds.

Recent findings

Studies on AIT for fungi were performed only for the two predominating mold species in the external environment, namely *Cladosporium* and *Alternaria*. There is no evidence for other mold species. Recent finding in the literature are lacking; the 2 most recent studies on AIT for molds were published in 2011. Overall, 13 studies were identified (the first was published in 1986), but only nine of these compared AIT to placebo. The studies are small (median study sample size, 27 patients) and of low quality, owing to several defects leading to moderate-to-high risk of bias. Symptoms improvement and medication use reduction, which are the main outcome measures of the studies, were inconsistently demonstrated. There are some concerns about safety with *Cladosporium* extracts, whereas vaccines with *Alternaria* extracts seem to be safe and well tolerated.

Summary

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

Keywords

Alternaria, asthma, *Cladosporium*, GRADE, rhino-conjunctivitis

INTRODUCTION

Allergy to fungi is a relatively common condition with a prevalence estimated about 5% of European patients with respiratory allergy [1,2]. *Alternaria*, the most important outdoor fungus together with *Cladosporium*, is the fifth most common sensitizing agent in Europe, after *Dermatophagoides*, *Graminaceae*, cat, and birch. The peak exposures are from May to August for *Cladosporium* spores and from July to September for *Alternaria*.

There is also some evidence on the role of indoor fungi in respiratory allergy, particularly *Aspergillus*, *Candida*, *Penicillium* [3], whereas data on other fungal species are lacking.

Symptoms of patients sensitized to outdoor fungi are similar to those presented by patients sensitized to aeroallergens, such as pollens, house dust mites, animal dander, and include rhino-conjunctivitis and asthma. Therefore, treatment options for these patients are the same as patients with sensitization to aeroallergens, including allergen immunotherapy (AIT). Unfortunately, studies assessing efficacy of AIT with mold extracts are very few. In this study, we will review the main studies on

AIT for molds in order to provide some evidence on the benefits and harms of this treatment.

ALLERGEN IMMUNOTHERAPY

The clinical efficacy and safety of AIT has been demonstrated by a number of studies [4–6]. Hay fever is the most common allergic disease studied. The majority of studies on AIT for grass pollen allergens concern rhino-conjunctivitis, whereas studies of asthma are lower in number. Both forms of AIT administration used in clinical practice, subcutaneous immunotherapy (SCIT) and sublingual

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KEY POINTS

- Using the GRADE approach, the evidence of clinical benefit of AIT for molds resulted low in strength, because of high level of inconsistency between the studies and their high risk of bias
- Allergenic extracts are not standardized, limiting the comparability between the study results.
- There are some safety concerns with *Cladosporium* extracts, whereas vaccines with *Alternaria* extracts seem to be safe and well tolerated.
- High-quality studies with an adequate sample size are needed.

immunotherapy (SLIT), have been extensively studied. Studies on SLIT are the newest and their overall quality is generally higher than SCIT studies.

PROBLEMS IN THE ASSESSMENT OF ALLERGEN IMMUNOTHERAPY EFFICACY: THE CHOICE OF THE OUTCOME MEASURE

Outcome measures can be subdivided into hard outcome measures, surrogate outcome measures, patient-related outcome measures (also known as 'soft outcome measures'), and composite outcome measures [7]. Patient-related outcome measures relate mostly to outcomes that are important to patients, such as reducing symptoms and improving quality of life. This kind of outcomes is the most frequently used in randomized controlled trials (RCTs) on AIT, particularly the 'symptom score' also called 'total rhino-conjunctivitis symptom score' (RTSS). The limit of this measure is that it is a subjective measure; in contrast, a hard endpoint is an endpoint that is well defined and can be objectively measured.

In some studies there can be some reason to use a surrogate outcome measure. In AIT studies this measure is the so-called medication score, expressing the number of medications used by patients as surrogate for clinical benefit, assuming that symptom reduction in subjects treated with AIT leads to a reduction of the use of symptomatic medications.

In AIT studies, the total combined symptom-medication score (TCSMS) is often used as composite outcome measure and, sometimes, it is also the primary outcome. Generally, the use of composite outcome measures is affected by various problems, which may complicate their interpretation. The main problem of this measure in AIT studies is that TCSMS is made up of the sum of two different scores (symptom score and medication score) that have

different outcomes (symptoms and medication use) and different scales. Therefore, computing this score rises some methodological concern [5,8].

Generally, in addition to other criteria used to judge the value of studies, such as the representativeness of the study sample or the randomization procedure and the use of blinding, it is useful to check the number of nonhard (morbidity-related) outcomes included in a composite outcome measure: the larger this number, the less certain one can be of its clinical relevance.

THE STUDY QUALITY AND THE GRADE APPROACH

The approach proposed by the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) working group provides a specific definition of the quality of evidence, which reflects the extent to which we are confident that an estimate of the effect is correct [9,10]. In other words, the results of different studies are taken into consideration as a whole and the evidence they generate must be evaluated in terms of reliability based on the quality of the individual studies and the overall agreement between the study results. According to this approach, specific factors have to be considered critical in determining the quality of the evidence. There are factors that can reduce the quality of the evidence such as: limitations in study design or execution (risk of bias); inconsistency of results (inconsistency of direction of the effect across studies for a given comparison and outcome); indirectness of evidence (surrogate outcome measures: the relevance of the collection of study data to the question of interest); imprecision; publication bias. In contrast, other factors can increase the quality of the evidence: large magnitude of effect; all plausible confounding would reduce the demonstrated effect or increase the effect if no effect was observed; dose-response gradient [9,10].

The evidence for the established outcome is graded as: high grade, that is high confidence that the evidence reflects the true effect; 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); 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); insufficient evidence. Observational studies without special strengths (i.e. large magnitude of an effect; dose-response gradient; effects of all plausible residual confounding accounted for) or with important limitations (i.e. study design or execution; publication

bias; imprecision; inconsistency) were considered to provide low quality of evidence.

STUDY QUALITY AND EVIDENCE OF ALLERGEN IMMUNOTHERAPY FOR FUNGI

As already mentioned, studies on benefits and harms of AIT for fungi are very few. A total of nine randomized studies comparing AIT to placebo or pharmacotherapy have been published (Table 1) [11[¶]]. These studies comparing AIT to placebo include a total of 268 patients (median study sample size, 27 participants) [12–20]. The evidence is limited to AIT with extracts of 2 outdoor fungi, namely *Cladosporium herbarum* and *Alternaria* (*alternata* and *tenuis*). The majority of the studies used SCIT (six of nine), two studies used SLIT, the remaining study used oral immunotherapy. Six out of the nine studies enrolled children (<18 years) with a median age of 12 years. The treatment duration was 12 months for seven of the nine studies, and 36 months for the remaining two studies. The cumulative dose of extract used varied greatly among the studies. The most commonly used outcome measures were symptom score and medication score, but diverse systems and scores for evaluating both primary and secondary outcomes were used (symptom score, medication score, visual analog scale, quality of life, bronchial/nasal/conjunctival provocation test results, peak expiratory flow, changes in serum specific IgE levels, changes in skin prick test results; Table 2). The comparators groups were placebo or pharmacotherapy [11[¶]].

The AIT evidence summary is reported in Table 3. There are only two studies published on AIT for *Cladosporium herbarum*, both performed in Sweden, Finland, and Denmark [12–13]. In these studies, published in 1986, AIT was administered by the subcutaneous route. Although these studies are double blind randomized placebo controlled studies (DBRPC), their small sample size (52 patients in total, including cases and controls) and their moderate-to-high risk of bias make the strength of the evidence low. The primary outcome measures used were symptom score and medication score. For both outcomes, one study was in favor to pharmacotherapy, whereas the other reported no statistically significant difference between active and placebo. The vaccine had generally a low tolerability, because 24 patients out of 27 in the active group manifested general reactions.

There are seven studies published on AIT for *Alternaria alternata*, four of which used SCIT [14–20] (Table 3). Only four of these were DBRPC, the remaining studies were observational studies without placebo in the control group. There was a

slight prevalence of studies in favor of AIT in terms of symptom reduction (four vs. three studies) and symptomatic medication use (four vs. three studies; Table 3). The benefit of AIT was more evident in studies with a longer follow-up. Also in the *Alternaria* studies, there is a moderate-to-high risk of bias, contributing to lower the strength of the evidence. Regarding safety and tolerability, there is evidence that *Alternaria* vaccines are safe and well tolerated. Only 13 of 105 patients in the active group of the seven studies reported general adverse reactions. Local reactions were negligible in number and severity (Table 4).

A very recent RCT provided further evidence on the efficacy and safety of AIT with a purified isoform of the *Alternaria* major antigen Alt a1 administered subcutaneously [21]. This form of AIT seems safe and well tolerated. Results of efficacy are limited by high rate of lost-to-follow up patients and withdrawals, which reduced by about 50% the patient population, analyzed for the main end-point. The exclusion of these patients from the comparison groups apparently generated groups with different baseline characteristics.

Finally, a controlled nonrandomized study performed on pediatric population showed a benefit of AIT with *Alternaria tenuis* compared to pharmacotherapy alone [22].

In addition to case-control studies, some additional evidence on the efficacy of SCIT for fungi is provided by two uncontrolled studies [23–24]: the first study assessed efficacy (nasal symptom and concomitant medication reduction) and safety of AIT for *Alternaria tenuis*, administered as SCIT and SLIT in 23 patients followed-up for 2 years [22]. Changes in outcome measures were assessed comparing the baseline to the follow-up period (same patients after 2 years of treatment). A statistically significant difference in favor of SLIT was shown for rhinitis symptom improvement and use of symptomatic drug reduction. These findings were confirmed by nasal provocation test results. SLIT displayed also a better safety profile compared to SCIT [23].

The second study, published in 2009, assessed the reduction of nasal rhinitis and asthma symptoms, the use of concomitant medications and quality of life in 99 patients after 1 year of treatment with AIT for *Alternaria alternata* compared to the baseline values. Despite the improvement in the outcomes because of AIT, 21 patients had to attend emergency department due to exacerbations of their allergic disease, and one of them was admitted to hospital [24].

Finally, the efficacy of AIT was also shown in allergic fungal sinusitis (AFS) [25]. There are no RCT

Table 1. Characteristics of the studies (previously published in [11*])

Study	Country	Study design	Allergen	AIT type	Patients (n)	Male (%)	Mean age (mean±SD)	ARC (%)	Asthma (%)	Treatment duration (months)	Major allergen	Cumulative dose
Dreborg 1986 (12)	Sweden Finland	DBRPC	<i>Cladosporium herbarum</i>	SCIT	AIT: 16 > 16 Pla: 14 > 14	46.6	11	NR	100	12	NR	508,880 BU
Malling 1986 (13)	Denmark Sweden	DBRPC	<i>Cladosporium herbarum</i>	SCIT	AIT: 11 > 11 Pla: 11 > 11	72.7	25 31	NR	100	12	NR	557,260 BU
Horst 1990 (14)	France	DBRPC	<i>Alternaria alternata</i>	SCIT	AIT: 13 > 13 Pla: 11 > 11	70.8	12.1±4.7 13.3±14.6	100	41.7	12	Alt a 1	58,850 BU (47.1 µg)
Criado Molina 2002 (15)	Spain	Randomized (no placebo)	<i>Alternaria alternata</i>	Oral IT	AIT: 22 > 16 PT: 22 > 16	59.4	12.4±3.3 12.3±3.2	84.4	90.6	12	Alt a 1	288,000 PNU
Tabar 2008 (16)	Spain	DBRPT	<i>Alternaria alternata</i>	SCIT	AIT: 14 > 13 Pla: 14 > 10	83.3	12.8±4.1 14.9±6.5	100	89.3	12	Alt a 1	20,483 UBE (1.23 µg)
Cortellini 2010 (17)	Italy	DBRPT	<i>Alternaria alternata</i>	SLIT	AIT: 15 > 15 Pla: 12 > 11	55.5	18.8±7.2 24.0±8.8	100	14.8	10	Alt a 1	60 µg
Pozzan 2010 (18)	Italy	SB ^a , randomized (no placebo)	<i>Alternaria alternata</i>	SLIT	AIT: 34 > 33 PT: 18 > 18	63.5	18±9 19±10	73.1	32.7	36	Alt a 1	129,6 µg 43.2 µg/yr
Kuna 2011 (19)	Poland	DBRPT	<i>Alternaria alternata</i>	SCIT	AIT: 30 > 25 Pla: 20 > 19	50	11.7±3.9 11.2±4.1	92	76	36	Alt a 1	123,000 TU (196.8 µg; 65.6 µg/yr)
Kilic 2011 (20)	Turkey	Randomized (no placebo)	<i>Alternaria alternata</i>	SCIT	AIT: 12 > 9 PT: 12 > 7	NR	10.2±2.2 10.1±2.1	NR	100	12	NR	NR

AIT, allergen immunotherapy; IT, immunotherapy; SD, standard deviation; ARC, allergic rhino-conjunctivitis; DB, double blind; SB, single blind; PC, placebo controlled; RCT, randomized controlled trial; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; Pla, placebo; PT, pharmacotherapy; BU, biological units; PNU, Protein Nitrogen Units; UBE, unidades biológicas equivalentes; TU, total units; *range; yr, year; NR, not reported. The symbol '>' separated the patients randomized (left) from the patients analyzed (right).

^aAssessor blinded.

Table 2. Summary of the outcomes assessed in clinical studies (unpublished)

Study	SS	MS	VAS	QoL	BPT	NPT	CPT	PEF	SPT	IgE	Spore count
Dreborg, 1986	x	x			x		x	x	x		x
Malling, 1986	x	x						x	x	x	x
Cantani, 1988	x										
Horst, 1990	x	x				x			x	x	
Criado Molina, 2002	x	x			x			x	x	x	
Tabar, 2008	x	x	x	x							
Cortellini, 2010	x	x								x	x
Pozzan, 2010		x	x								
Kuna, 2011	x		X	x		x					x
Kilic, 2011	x	x	x	x	x			x		x	x

BPT, bronchial provocation test; CPT conjunctival provocation test; IgE, serum specific IgE levels; MS, medication score; NPT, nasal provocation test; PEF, peak expiratory flow; SPT, changes in skin prick test results; SS, symptom score; VAS, visual analog scale; QoL, Quality of Life.

Table 3. AIT evidence summary (previously published in [11^a])

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> (12, 13) - <i>Alternaria</i> (14-20)	Four studies had a high risk of bias (13, 15, 18, 20). Four studies had a moderate risk of bias (12, 14, 16, 17). One study had a low risk of bias (19). 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 favor of AIT (14, 17-19 ^a), 2 of which showing a large magnitude of effect (14, 17). Five studies reported no statistically significant differences (12, 13, 15, 16, 20).	Low	The quality of evidence was downgraded by two levels due to high level of inconsistency: five studies in favor of AIT; five negative or null. Meta-analysis including three out of the nine studies showed a considerable (i.e. $I^2 > 75\%$) level of heterogeneity ($I^2 = 78\%$) between the study results (11). The quality of evidence can also be downgraded by two levels due to imprecision, since CIs around estimates of effect are wide, including both appreciable benefit and appreciable harm (11). <LBREAK"/>Moreover, the studies include relatively few patients.
Medication use	268 - AIT, 151 - PT, 117	9	<i>Cladosporium</i> (12, 13) <i>Alternaria</i> (14-20)	Four studies had a high risk of bias (13, 15, 18, 20). Four studies had a moderate risk of bias (12, 14, 16, 17). One study had a low risk of bias (19). 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 favor of AIT (14, 17-19), 2 of which showing a large magnitude of effect (14, 17). Five studies reported no statistically significant differences (12, 13, 15, 16, 20).	Low	The quality of evidence was downgraded by two levels due to high level of inconsistency: four studies in favor of AIT; five negative or null. Meta-analysis including six out of the nine studies showed a considerable (i.e. $I^2 > 75\%$) level of heterogeneity ($I^2 > 94\%$) between the study results (11). The quality of evidence can also be downgraded by two levels due to imprecision, since CIs around estimates of effect are wide (11). Moreover, the studies include relatively few patients.

Abbreviations: AIT, allergen-specific immunotherapy; PT, pharmacotherapy; RCT, randomized controlled trial; SS, symptom score.

^aThe Pozzan study was in favor of AIT, but used VAS as the measure of efficacy, instead of SS.

Table 4. Safety data (previously published in [11^a])

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

Abbreviations: NR, not reported; PT, pharmacotherapy.

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

on the efficacy of AIT for AFS, but some evidence was provided by uncontrolled studies using SCIT and, recently, also by a small study including 10 patients treated with SLIT. Although further investigations are needed to confirm the role of AIT in this disease, the findings merit attention, considering that the cornerstone of AFS treatment, that is surgery followed by medical treatment, is not sufficient in most cases to cure the disease, which shows a high relapse rate.

LIMITATIONS OF THE STUDIES ON ALLERGEN IMMUNOTHERAPY FOR FUNGI

Evidence supporting the administration of immunotherapy for *Alternaria* and *Cladosporium* is of low strength, because of a number of reasons. First, the overall risk of bias of the few available studies was moderate-to-high. Although the majority of the studies analyzed were DBRPC, several sources of bias, such as the unclear description of the random sequence generation [12,15,18] and allocation concealment, [12,13,15,20] and the lack of blinding [12,18,20] greatly reduces their overall quality. Furthermore, in four of the nine randomized studies, there was the participation of sponsor companies in the study design and the interpretation of data [13,16–18]. As it was repeatedly shown, these studies are more likely to report results that favor the

sponsor's product than studies supported by independent sources [26–27].

Besides the study quality, we encountered another problem in the assessment of the overall AIT benefit, which is the heterogeneity in study design and conduct (double-blinded or single-blinded, open parallel design, nonpaired controls, evaluation at different time points, per protocol or intention to treat evaluation, and so on). 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. These problems limit the comparability between the study results and consequently an overall assessment of treatment benefit.

These considerations are also valid for the 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 40 times greater [19] than the lowest [16] (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.

Interestingly, the studies with longer treatment duration 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. However, the overall small number of studies and their small sample size make it impossible to draw any meaningful conclusion based on these latter findings.

Regarding safety, studies included in this analysis did not report on safety data in a uniform and consistent manner. There was no report of life-threatening adverse events, in this review (Table 3). 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, because of their difference in measure units. Moreover, safety data on *Cladosporium* were drawn from studies performed more than 30 years ago [12,13]. More updated reports on this issue would be needed, because no other study on *Cladosporium* has been performed since then.

CONCLUSION

In conclusion, we found low strength in the evidence in supporting the use of AIT for molds. 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. Because of 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 [4]. 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. Finally, there is a need for standardization of allergenic mold extracts. Carrier-bound peptides without allergenic activity should also be considered [28]. Until then, AIT for molds will remain an

uncommon method for treating respiratory diseases because of fungal sensitization.

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Conflicts of interest

There are no conflicts of interest.

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- of special interest
- of outstanding interest

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