

Allergen-Specific Immunotherapy: Which Outcome Measures are Useful in Monitoring Clinical Trials?

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- Quality of life • Specific immunotherapy • Responder-analysis
- Allergen challenge

Sneezing, rhinorrhea, itching, nasal obstruction, and sleep disturbance are typical symptoms of allergic rhinitis (AR). These symptoms may impair patients' daily abilities as well as their general well-being and work productivity.¹ Current treatments of AR include allergen elimination, pharmacologic treatment, and specific immunotherapy (SIT). A revision of the Allergic Rhinitis and its Impact on Asthma (ARIA) guideline,

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aimed at providing systematically and transparently developed recommendations about the 3 therapeutic options in AR, has been published.² Specific subcutaneous immunotherapy (SCIT) and specific sublingual immunotherapy (SLIT) represent the only immune-modifying and causal treatments available for allergic patients.^{1,3} The evidence for the effects of SIT is based on controlled and randomized clinical trials using specific primary and secondary outcome measures (end points).^{4,5}

Primary end points for evaluating clinical outcome are the severity of symptoms and the need for concomitant medication, and are usually obtained on a daily basis by keeping diaries.⁶ Secondary end points may include the specific and general (generic) quality of life (QoL)⁷ or impact on work-related abilities,⁸ and are usually obtained by questionnaires at follow-up. Some trials have also included allergen provocation tests, allergen chambers, or other surrogate markers such as allergen-specific IgG₄, the ratio of IgE to IgG₄, the IgE-binding capacity of the patient's serum, cytokine analysis, cell-activation markers, the number or differentiation of immune cells, and cell proliferation assays, but these are not considered to be suitable parameters for primary assessment. However, they might be used for investigating the immunologic mechanisms of SIT.^{9,10}

This article gives an overview of approved and widely used methods of monitoring the clinical outcome of SCIT and SLIT in both clinical trials and daily routine. In 2000, a draft guidance document on outcome parameters was provided by the US Department of Health and Human Services Food and Drug Administration (FDA).¹¹ More recently, the European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) guideline on the clinical development of products for SIT has outlined recommendations in assessing outcome variables in clinical trials.¹² It must be noted, however, that universally accepted and authoritative national and international guidelines are still lacking with respect to the assessment of the therapeutic effects of SIT.⁶

PATIENTS' SELF-RATED DIARIES: VALUABLE TOOLS IN ASSESSING PRIMARY END POINTS

Successful SIT results in a decrease in both symptom severity and the need for concomitant medication, which can be monitored by daily entries in patients' diaries. In 2007, a task force of the World Allergy Organization (WAO) proposed a model for standardization of SIT clinical trials stipulating that patient-rated scores should be preferred as primary outcome parameters.⁶ The individual scores can be recorded instantaneously (ie, an evaluation of symptom severity immediately before the next dose) and reflectively (predefined time period such as 12 hours).

However, this requires a compliant and motivated patient. The design of the diary depends very much on the experience of the allergologist concerned. According to the authors' experience, some practical aspects recommended for a diary to be a reliable and reproducible source of data can be summarized thus^{5,13}:

1. Clear and easy to understand instructions
2. Small and handy size to be carried around easily
3. Only one page for each day
4. Large print for elderly patients
5. Waterproofed bindings
6. Multiple-choice questions as well as some space for free text.

As an alternative to paper diaries, electronic tools (eg, handheld computer or Internet-based devices) might be used in clinical trials, with the advantage that timely

and appropriate entries by the patients can be verified. On the other hand, electronic diaries require well-trained and experienced patients and additional logistic expenditures.

PRIMARY END POINTS IN CLINICAL TRIALS: SYMPTOMS AND CONCOMITANT MEDICATION

Following the guidance in *Good Clinical Practice: Consolidated Guideline* (ICH E6) adopted by the ICH, May 1, 1996, clinical trials should include primary parameters “capable of providing the most clinically relevant and convincing evidence directly related to the primary objective of the trial.”¹⁴ However, in the 2008 *Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases* the European Medicines Agency (EMA) states that to date, there are no symptoms scores thoroughly validated for clinical trials on SIT.¹² However, for the evaluation of AR the 4 individual nasal symptoms “congestion,” “sneezing,” “itching,” and “secretion,” and 2 conjunctival symptoms “itching of eyes” and “ocular secretions,” are generally accepted. These symptoms are scored by patients on the diary card using a 4-point rating scale between 0 (no symptoms) and 3 (severe symptoms) so that the (combined) sum of the 6 individual symptom scores for a given day results in a rhinoconjunctivitis total symptom score (RTSS) ranging between 0 and 18 points (**Table 1**).¹²

The average RTSS (ARTSS) is then defined as the average of the daily RTSS during the observation period, for example, the peak pollen season.¹⁵ The RTSS was included in several recent trials on both SCIT¹⁶ and SLIT.^{17–19}

Shortness of breath, cough, wheezing, and chest tightness may also be considered in patients with concomitant lower airway symptoms.⁶ Hence, in other studies a total of 9 types of symptoms were rated in a combined symptom score of 3 nasal symptoms (running, blockage, sneeze), 3 ocular symptoms (itching, redness, tearing), and 3 bronchial symptoms (cough, wheezing, asthma/dyspnoe). Each symptom was scored on a scale of 0 to 3 and totaled daily, allowing a maximum score of 27 points.^{20,21} Another trial on SLIT included 4 nasal symptoms together with 3 conjunctival and 3 bronchial symptoms, resulting in a maximum score of 30 points^{22,23} and in trials on SLIT with tablets on both grass pollen allergic children/adolescents^{24,25} and adults^{26,27} 4 nasal together with 2 conjunctival symptoms (maximum: 18 points) and separately 4 bronchial symptoms (maximum: 12 points) were analyzed.

Fig. 1 displays examples of 6 different scoring systems that are used at present by some European allergen manufacturers.

Table 1 Evaluation of nasal symptoms using a 4-digit score	
0	No sign/symptom evident
1	Mild symptoms (sign/symptom clearly present, but minimal awareness; easily tolerated)
2	Moderate symptoms (definite awareness of sign/symptom that is bothersome but tolerable)
3	Severe symptoms (sign/symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping)

Data from Committee for Medicinal Products for Human Use (CHMP). Guideline on the clinical development of products for specific immunotherapy for the treatment of allergic diseases. CHMP/EWP/18504/2006. London; 2008; and Guidance for Industry. Allergic rhinitis: clinical development programs for drug products. Draft guidance. U.S. Department of Health and Human Services, Food and Drug Administration. Center for Drug Evaluation and Research; 2000.

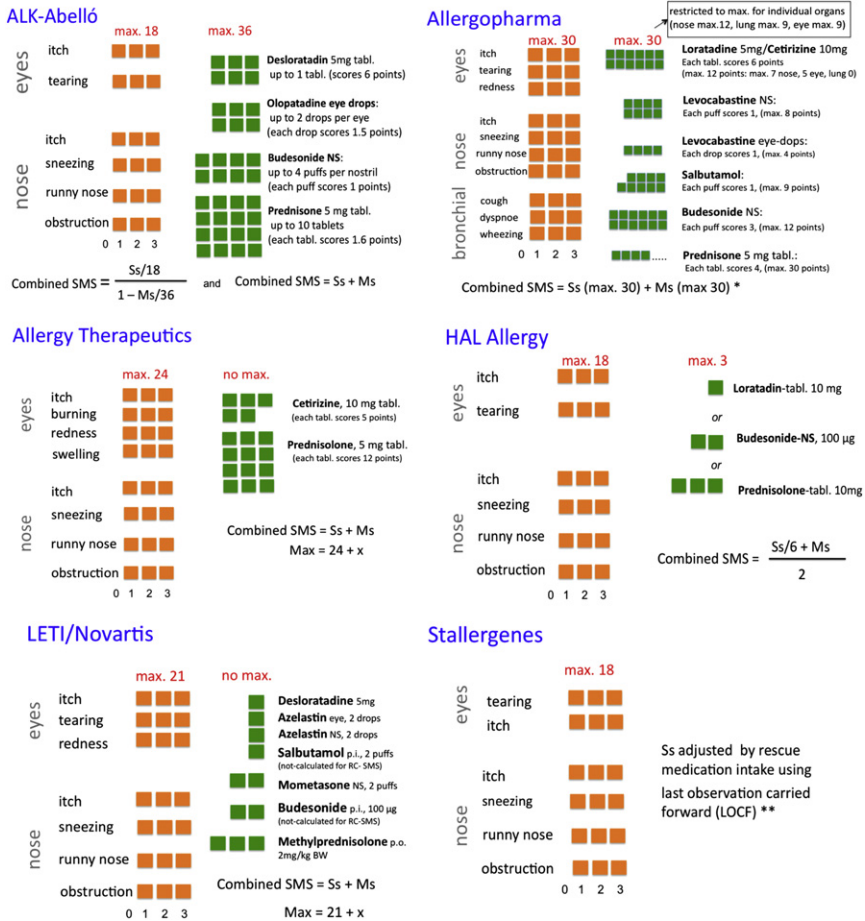


Fig. 1. Examples of currently applied symptom and medication score systems from different European allergen manufacturers. Allergic symptoms (orange box = 1 score point) and rescue medication scoring (green box = 1 score point) are given as well as the way symptom score (Ss) and medication score (Ms) are weighted for a combined symptom-medication score (SMS). Some medication scores do not restrict the entry in the case of multiple drug doses leaving the score open for extraordinarily high values (no max.). top, topical; NS, nasal spray; RC, rhinoconjunctivitis. * Based on this combined SMS the "Allergy-Control-Score (ACS)" has recently been validated.²⁹ ** The "average Adjusted Symptom Score" (AdSS) is derived from the RTSS using "last-observation-carried-forward" (LOCF).³⁰

In SIT clinical trials, symptom and medication scores are often assessed independently, with defining symptom scores regarded as primary efficacy end points (eg, RTSS) while use of concomitant medication defined as a secondary outcome parameter.¹⁵ However, there is a complex and multifactorial interaction between symptom scores and the use of concomitant medication. First, the use of concomitant antiallergic medication results in reduction of clinical symptoms of AR, and thereby alleviates the daily symptom scores in both active and placebo-treated patients. Second, placebo-treated patients are expected to use concomitant medication in a higher extent than patients in the active group. Hence, if the assessment of efficacy

is estimated on the symptom scores alone, the use of rescue medication will alleviate the superiority of active over placebo treatment.¹⁵

Because of these interdependences, a task force of the WAO suggested a model for standardization of clinical trials on SIT. The task force recommended that symptom scores always be combined with the concomitant medication scores to provide clinically more relevant information on SIT than assessing each of these two outcome parameters separately⁶; this is achievable by a combined score (weighted sum scores of symptoms and rescue medications)²⁸ or a symptom score adjusted for the use of medication, using a predefined algorithm.⁴ However, to date no standardized method for combined scores has been accepted.^{6,15}

Following the WAO guidelines, nasal, conjunctival, and oral antihistamines should be weighted with a score of 1, inhalative or nasal corticosteroids with a score of 2, and the daily administration of oral corticosteroids with a score of 3 points. The “symptom load” of each day is then evaluated by equally adding the sum of the symptom score to the medication score, aiming to reproduce a weighted balance between use of concomitant medication and the extent of symptoms.⁶ However, there is a high variability between studies on SIT of how concomitant medication is scored, as topically and systemically applied rescue medication is differently weighted (see **Fig. 1**). Comparison of these studies suggests that the symptom and medication scores currently used in clinical trials as primary outcome parameters are not standardized and give variable results.

Recently, a novel combined symptom-medication score (Allergy-Control-SCORE [ACS] [TM]) has been formally validated for the first time in a clinical trial on 81 patients with allergic rhino-conjunctivitis and/or asthma and 40 healthy controls by comparing this score to global allergy severity, quality of life, and allergy-related medical consultations.²⁹ Moreover, the average Adjusted Symptom Score (AdSS) has been calculated in a post-hoc analysis of a Phase IIb/III trial on 628 grass pollen allergic adults¹⁷ and a Phase III trial on 278 paediatric patients¹⁸ and has been recently published giving extensive formula for exact calculation of this score.³⁰ It adjusts the symptom for the use of medication by calculating the “last observation carried forward”, which has been frequently applied to handle missing data in clinical trials.¹⁵ In the case that the patient is taking concomitant medication, the last (most recent) RTSS score of the day before medication has been started is calculated for further analysis. This AdSS-score is carried forward until the day the patient stops antiallergic medication again.

It is obvious that the clinical outcome should be taken into account for the final interpretation of the clinical efficacy of SIT (“nonresponder” vs “responder”), that is, the decrease in symptom scores should be high enough to clearly reduce allergy-related morbidity under “real life” conditions.³¹ In some clinical trials, more emphasis is placed on the statistical significance of changes rather than their clinical relevance, but this does not always illustrate the clinical benefit of a therapy in practice. For example, a 15% reduction in combined symptom-medication scores might reveal statistical significance, but this does not necessarily mean a clinical benefit for the patient per se. Therefore, ranges of improvements of symptom or medication scores for discriminating between effective and noneffective therapy in SIT have been proposed by Mallin.³²

1. No effect, improvement of <30%
2. Little effect, improvement of 30%–44%
3. Moderate effect, improvement of 45%–59%
4. Strong effect, improvement of ≥60%.

Although this classification seems arbitrary, it has served as a simplified “rule of thumb” in assessing “relevant” effects of therapy in SIT trials.

Assessing the Relevant (Pollen-)Allergen Exposition

In pollen allergies, it is standard to measure pollen concentrations in a defined volume of air (eg, using Burkard volumetric spore traps). Pollen counts are usually expressed as the mean daily number of pollen grains per cubic meter of filtered air. Usually a limited time interval is defined as the time frame for the evaluations. This interval may be the “time of peak pollen season” or the “relevant pollen season,” or others. An exact definition is still lacking.⁶ In one approach, a certain number of pollen grains per cubic meter is predefined and the “peak pollen season” or “relevant pollen season” and starts per definition when this number is reached, for example, once or on more than 3 consecutive days. The season ends, vice versa, if the pollen count falls below this value in the same way. However, there is the question of the “relevant” pollen exposure and defining threshold values for this. In a double-blind, placebo-controlled (DBPC) trial on 184 tree pollen-allergic patients receiving SCIT with a depigmented polymerized tree pollen extract, Pfaar and colleagues³³ analyzed symptom and medication scores on days above a series of different threshold values for pollen exposure (≥ 250 , ≥ 300 , ≥ 400 , ≥ 500 grains/m³ per 24 hours). A clear relationship between pollen exposure and the magnitude of combined symptom and medication score caused by this exposure, as well as differences for the size of response to treatment for different thresholds, were demonstrated. Further studies are necessary to define more precisely the optimal threshold value for pollen exposure and, therefore, the “relevant” time frame for evaluation.

A different approach is to retrospectively evaluate the time with the maximum pollen count over a certain time frame, for example, 4 weeks. Rarely, a complete analysis of the entire pollen season will be performed. However, the latter approach would more likely reflect the expectations of the patient in real life, who would like to achieve relief during the entire pollen season and not only during an arbitrarily defined peak period.

Analyzing Different Pollen Counts in Different Areas

In multicenter trials it is necessary to take into account the different pollen counts in different areas. One model is to choose several pollen traps nearest to the study centers, possibly within the same area. However, it is well known that the “real” allergen exposition of the individual patient cannot be detected in this way. The duration and intensity of the pollen season is expected to be variable and, therefore, pollen counts are conducted at least 3 times each week in the vicinity of each center. However, as each study center could recruit subjects from a wide area, this approach may not necessarily identify the most appropriate peak weeks of the pollen season for individual subjects. A previous study investigating the allergic response to birch pollen highlighted that some subjects report severe symptoms even on days with low birch pollen levels.^{34,35}

While a recent analysis by Buters and colleagues³⁶ demonstrated that although, in general, pollen count and allergen levels overlapped, in some cases the difference between estimated allergen exposure based on pollen counts and determination of allergen levels by immunoassay could vary by as much as a factor of 10. The investigators explained this variance by demonstrating a large increase in expression of the birch pollen allergen Bet v 1 just before pollination. Consequently, the day the pollen is released during ripening will affect its potency. These results suggest that even in locations close to the pollen trap, pollen counting may not be the most appropriate measure of allergen exposure.

The same applies for indoor allergens such as house dust mites and cat allergens. Here, assessments of allergen exposure by measuring major allergens in reservoir dust samples (bed, carpet, soft furnishings, and so forth) using monoclonal antibody-based enzyme-linked immunosorbent assays might provide levels of individual exposure. The World Health Organization/International Union of Immunological Societies Allergen Standardization Committee has initiated a multicenter project to develop international standards for purified allergens. This program had been coordinated through the European Union CREATE project (Certified Reference Materials for Allergenic Products).^{37,38}

Individual pollen samplers are an alternative that has been used to assess pollen, mites, and cat allergens.^{39,40}

Peak Placebo Symptom Days

Another approach to evaluating the relevant pollen exposure in allergic individuals is to identify the peak symptom days for the 4 weeks with the highest symptom medication score (SMS) during the pollen season, based on the findings in the placebo group.⁴¹ These “peak placebo symptom days” are not necessarily consecutive and vary between centers. It is assumed that the period when the SMS in the placebo group of the specific study center is at its highest indicates the heaviest pollen load. This novel approach allows the local allergy burden to be determined at different centers. However, representing a postrandomization variable, leading to a post hoc analysis, this attempt would not be valid for the design of a controlled prospective study.

Responder Analysis and Baseline Year

The current European Union regulatory bodies (EMA) recommend that a responder analysis should be performed in addition to assessing efficacy on the basis of SMS scores alone.¹² In this guideline “responders” are defined as “patients with a combined score below a pre-specified level indicating a clinical benefit for the patient.” However, for this analysis a “baseline period” assessing the patient’s symptom load one pollen season before randomization (and treatment) is required. Due to the unpredictability and variability of allergen exposure to pollen exposure (see earlier discussion), baseline pollen seasons are not generally useful for this pretreatment/posttreatment analysis.⁶ Moreover, this procedure is time-consuming and expensive.⁶

For this reason, a “modified responder analysis” has been recently described, setting the median value for the primary outcome using area under the curve (AUC) for SMS for the entire pollen season for the placebo group as “baseline,” and analyzing numbers of responders and nonresponders at different levels of reduction in the combined scores from this baseline.³³ By the use of receiver-operator curves (ROC), the authors found a cutoff of 30% to optimally discriminate between active and placebo-treated patients during the entire season. These results are in line with the “Malling definition” of a clinically relevant treatment effect of SIT of at least 30% (see earlier discussion³²).

Visual Analog Scales

Another self-assessment method, the visual analog scale (VAS) or visual rating scale (VRS), has been recommended for psychometric analysis.^{6,12} VAS has been shown to be useful in the monitoring of the severity of symptoms, especially for long-term follow-up in the treatment of perennial AR, for example, comparison of baseline- and posttreatment conditions. Patients grade their symptoms retrospectively (eg, in the last week or in the last month) by putting a vertical line on a 10-cm line representing the severity of symptoms from 0 = “no symptoms” to 10 = “very severe symptoms.”

Thereby, one advantage of the VAS is through the use of a continuous variable rather than an ordinate scale. One the other hand this narrows the spectrum of the responses, as patients tend to avoid marking the extremes of the VAS.⁷ However, as secondary outcome parameters VAS have been widely used in different SIT trials and have supplemented other outcome variables such as symptom and medication scores.^{16,22,42,43}

SECONDARY END POINTS IN CLINICAL TRIALS: HEALTH-RELATED QUALITY OF LIFE

The impact of AR on the patient’s health status goes far beyond the organ-specific (nasal, conjunctival, and bronchial) symptoms alone.⁵ Thus, the objective of a clinical trial of SIT should not simply be to monitor certain organ-related symptoms but to additionally evaluate the patient’s mental and physical condition, the so-called health-related quality of life (HRQoL).⁴⁴ The impairment of allergic patient’s physical fitness, school and occupational achievements, life satisfaction, and “day-to-day activities” has been clearly evaluated in many clinical trials. Moreover, reports have revealed that sleep impairment results in a noticeable reduction in general activities and diminished HRQoL of patients with AR.⁴⁵ Therefore, assessing the HRQoL is a valuable and sensitive tool for evaluating a therapeutic improvement of SIT as a secondary outcome, with symptom and medication scores as the primary outcome parameters. Two types of HRQoL questionnaires are available: generic and disease-specific (Table 2).

Generic QoL Questionnaires

First, there are general health-related (generic) questionnaires available, which enable patients with different diseases to be compared on the basis of their QoL from the patient’s point of view. In other words, these generic QoL questionnaires compare

Table 2 Generic and disease-specific QoL questionnaires for specific immunotherapy trials	
Generic (Not Disease Related)	
Medical Outcome Study Short Form-36	Stewart et al, ⁴⁶ Ware and Sherbourne ⁴⁷
Medical Outcome Study Short Form-20	Carver et al ⁵²
Munich Life Dimension List (MLDL)	Kremer et al ⁵⁴
Work Productivity and Activity Impairment Questionnaire (WPAI)	Prasad et al ⁸
Specific (Disease Related)	
Asthma Quality of Life Questionnaire	Juniper et al ⁹⁸
Mini Asthma Quality of Life Questionnaire	Juniper et al ⁹⁹
Asthma Questionnaire 30	Barley et al ¹⁰⁰
Asthma Questionnaire 20	Barley et al ¹⁰⁰
Rhinoconjunctivitis Quality of Life Questionnaire	Juniper and Guyatt ⁵⁵
Mini-Rhinoconjunctivitis Quality of Life Questionnaire	Juniper et al ⁵⁶
Pediatric Rhinoconjunctivitis Quality of Life Questionnaire	Juniper et al ⁵⁸
Rhinasthma	Baiardini et al ⁶³
Rhinasthma (adapted German version)	Mosges et al ⁶⁴

Data from Canonica GW, Baena-Cagnani CE, Bousquet J, 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;62:317–24.

the burden of the different diseases, such as asthma or rhinitis, utilizing the identical questionnaire tool.⁷

To date, the best validated and most commonly applied generic questionnaire is the Health Status Questionnaire Short Form-36 (SF-36) (Table 3,^{46,47}). In 1994, Bousquet and colleagues⁴⁸ confirmed the SF-36 as a valuable tool in discriminating between patients with perennial AR and healthy subjects. It has also been validated by other groups.⁴⁹

This questionnaire comprises 36 questions in 9 health domains, and renders mental component summary (MCS) scores as well as a psychometrically based physical component summary (PCS).^{50,51} A modified version (SF-36 v2) has been translated into more than 120 languages in both standard 4-week and acute 1-week recall periods (detailed information on www.sf-36.org).

Other generic QoL questionnaires are the Medical Outcome Study Short Form-20 (SF-20),⁵² the Satisfaction Profile (SAT-P),⁵³ and the Munich Life Dimension List.⁵⁴ One additional example is the Work Productivity and Activity Impairment Questionnaire (WPAI), one of the most frequently used instruments in assessing the extent of the patients' occupational impairment caused by the disease.⁸

Taken together, the generic QoL questionnaires have the advantage that the impact of illness on the individual's quality of life can be globally assessed independently from the specific, underlying disease. Consequently the questionnaires have to be broad in

Table 3
Definition of health concepts with the SF-36 questionnaire

Measure	Number of Items	Definition
Functional Status		
Physical functioning	10	Extent to which health interferes with a variety of activities (eg, sports, carrying groceries, climbing of stairs, and walking)
Social functioning	2	Extent to which health interferes with normal social activities (eg, visiting with friends during past month)
Role limitations attributed to physical problems	4	Extent to which health interferes with usual daily activities (eg, accomplished less than would like)
Role limitations attributed to emotional problems	3	Extent to which health interferes with usual daily social activities (eg, accomplished less than would like)
Well-Being		
Mental health	5	General mood or affect, including depression, anxiety, and psychological well-being during the past month
Energy/fatigue	4	Tiredness, energy level
Pain	2	Extent of bodily pain in past 4 weeks
Overall Evaluation of Health		
General perception of health	5	Overall rating of current health in general
Change of health ^a	1	Evolution of general perception of health

^a Not included in (SF-36 v2).

Data from Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992;30(6):473–83; and Bousquet J, Bullinger M, Fayol C, et al. Assessment of quality of life in patients with perennial allergic rhinitis with the French version of the SF-36 Health Status Questionnaire. *J Allergy Clin Immunol* 1994;94(2 Pt 1):182–8.

their comprehensiveness, but this results in very little depth of assessment.⁷ Therefore in many conditions, such as rhinitis, generic QoL questionnaires are quite insensitive and often unresponsive to changes that might be small but undoubtedly essential to the patient's QoL. This disadvantage limits the usage of generic QoL questionnaires as tools in clinical trials on SIT, where the effects of the treatment on a homogeneous group of allergic patients are measured. As a consequence, disease-specific measures are more valuable tools in finding small, but clinically relevant changes in the patients' QoL in clinical trials on SIT.⁷

Disease-Specific QoL Questionnaires for AR and Asthma

HRQoL can be assessed on the basis of specific disease-related questionnaires, such as the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ),⁵⁵ which was validated in 1991 by Juniper and Guyatt, based on how symptoms and treatment affect the patient's social, emotional, and physical well-being. The RQLQ consists of 28 questions/items divided across 7 "main factors"/domains (activity limitations, emotional function, eye symptoms, non-hay fever symptoms, nasal symptoms, practical problems, and sleep problems), all of which are scored by the patients using a 7-point Likert scale ranging from 0 (ie, not troubled/none of the time) to 6 (ie, extremely troubled/all the time).⁵⁵ The 7 domains that impair the patient's QoL with AR as assessed by the RQLQ are as follows⁵⁵:

1. Practical problems: 3 items
2. Activities: 3 items
3. Sleep problems: 3 items
4. Emotional condition: 4 items
5. Nasal symptoms: 4 items
6. Eye symptoms: 4 items
7. Non-nasal/eye symptoms: 7 items.

For the domain "sleep problems" these items refer to "difficulty getting to sleep," "waking up during the night," and "lack of a good night's sleep." For the domain "activities" 3 items can be selected out of a list of 29 activities freely by the patient. These activities should be the 3 considered by the patient to be most affected by the disease.

The mean value for overall (global) RQLQ including all domains is calculated, as well as the mean value of each domain. The higher the score, the higher is the impairment of QoL. In controlled clinical trials a difference of 0.5 or greater in RQLQ domains between active and placebo-treated patients is regarded as clinically relevant.⁵⁵

A shortened version of this RQLQ, the Mini Rhinoconjunctivitis Quality of Life Questionnaire (Mini RQLQ), with 14 questions spanning the 5 domains "nose symptoms," "eye symptoms," "other symptoms," "practical problems," and "activity limitations," has been recently validated by the same group.⁵⁶ Furthermore, the RQLQ has been modified and adapted to different age groups of patients, taking into account that the effect on QoL may be different in allergic children and adolescents from that in adult allergic patients.^{7,57,58}

Several clinical studies have investigated the effect of antiallergic medication on QoL in allergic patients. A recent trial by Ciprandi and colleagues⁵⁹ on 123 patients with persistent AR clearly revealed a close relationship between QoL as measured by the RQLQ and clinical parameters such as nasal flow and eye symptoms, as well as immunologic parameters and functional surrogate markers. The investigators concluded that QoL is strictly related to the allergic inflammation process, and thereby might be used as a valuable leveling parameter in managing patients with AR.

Furthermore, RQLQ scores as secondary outcome measures have been assessed in several clinical trials on both SLIT^{60–62} and SCIT.^{16,20} In a dose-finding study with grass-allergen tablets containing timothy grass (*Phleum pratense*), overall RQLQ scores were significantly improved compared with placebo in the high-dose group (75,000 standardized quality tablet units daily), both at the first and second grass pollen season.⁶² A recent study on SLIT with grass-allergen tablets containing 5 grass allergens demonstrated that the RQLQ scores were improved in active treated patients, as were both primary (RTSS) and other secondary parameters (efficacy at pollen peak, combined scores, and immunologic changes).⁶⁰ RQLQ was also measured in a recent DBPC trial on grass tablets, with significant improvements in both RQLQ overall and all-domain scores (except “emotional function”) in the peak pollen seasons, not only in the 3-year course of therapy but also sustained in a follow-up year.⁶¹ In another DBPC dose-finding SCIT trial the effectiveness of 2 doses of an unmodified grass pollen extract in patients with moderately severe seasonal AR was investigated, and a clear dose-related response was found for the primary outcome parameter, symptom and medication scores.¹⁶ Of interest, a significantly better RQLQ as compared with placebo in 5 of the 7 RQLQ domains in the high-dose group (maintenance dose: 100,000 standardized quality units [SQ-U]) and only in 1 of the 7 RQLQ domains in the low-dose group (maintenance dose 10,000 SQ-U) was also revealed. Another SCIT trial using a mixture of 5 recombinant grass pollen allergens found that in addition to a significant improvement in combined symptom and medication scores in the first grass pollen season, SCIT resulted in a significant improvement of 5 of the RQLQ subdomains and of the global RQLQ in the second grass pollen season.²⁰

The specific disease-related questionnaire RQLQ only evaluates QoL as related to AR symptoms but not to allergic asthma, although both diseases are linked and often show comorbidity (concept of “united airways”). Therefore, the “Rhinasthma” questionnaire was validated by Baiardini and colleagues⁶³ for assessing the functional, physical, and emotional status of adult patients as influenced by AR and bronchial hyperreactivity. The German adapted Rhinasthma questionnaire is composed of 42 patients’ self-rated items and, more than other RQLQ questionnaires, considers the impact on AR on the social interactions of the patients.⁶⁴

Defining “Well Days” as New Outcome-Parameters

In addition to symptom scores and the use of antiallergic medication, the EMA recommends evaluating “days with sufficient symptom control.”¹² According to the EMA guidelines, these are defined as “days without intake of rescue medication and a symptom score below a pre-defined and clinically justified threshold.”¹² In recent years, this parameter has been increasingly included in several clinical studies on SLIT.^{24,42,65} However, the definition of the “clinically justified threshold” symptom score varies between different studies:

In a DBPC study on SLIT including 89 grass pollen-allergic patients, a 42-day period during the grass pollen season was analyzed.⁶⁵ During this 42-day period, “well days” were defined as days without any medication intake and with a symptom score of 4 or less. After 18 months of SLIT, actively treated patients reported a significantly higher median number of “well days” (52.4%) than did the placebo group (10.7%). These results corresponded well to results in the combined SMS scores.

In another DBPC study on SLIT with grass tablets, that included 253 children, aged 5 to 16 years, with grass pollen-induced rhinoconjunctivitis with or without asthma, “well days” were defined as days without any intake of concomitant medication and with a rhinoconjunctivitis symptom score of 2 or less.²⁴ The active group reported

53% well days whereas the placebo group reported 42% well days, with the difference being significant. Again, this result was in line with the study's other primary and secondary end points.

A large DBPC dose-finding study on SLIT with grass tablets by Didier and colleagues¹⁷ measured "symptom-free days" (%) during the pollen season. These days were defined when "0" (absence of symptoms) was recorded for each of the 6 rhinoconjunctivitis symptoms. In accordance with the differences in RTSS scores between the active groups and the placebo group and other outcome parameters, differences in the percentage of the symptom-free days were also shown.

Provocation Tests as Surrogate Markers

In its 2008 *Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases* the EMA emphasized that "provocation tests (eg, conjunctival, nasal or bronchial provocation or allergen exposure in allergen challenge chambers) and/or clinical end points may be used as primary end points" in early-stage, dose-ranging (phase 2) studies.¹²

The nasal provocation test (NPT) is a diagnostic tool used to confirm the clinical relevance of inhalative allergens for AR.^{66,67} This method is standardized, and provides both high sensitivity and high specificity.⁶⁸ It has been used in several clinical trials on SIT to reveal changes of the allergen-specific organ-related reactivity after treatment.^{69,70} For the latter, NPT should be performed with increasing threshold concentrations and the response compared with the results at baseline. In a controlled open trial of 53 grass pollen-allergic children, SCIT with a chemically modified preparation resulted in a 10-fold higher allergen concentration required to provoke a positive test result compared with pretreatment.⁷¹ In a SCIT-study with a short-term regimen of 7 preseasonal injections over 6 weeks, a significant increase in the dose that provoked a positive response in nasal allergen challenge was clearly demonstrated in active treated patients compared with patients on placebo.⁷²

The NPT with increasing threshold concentrations of allergens was also performed in a study on intralymphatic allergen administration (ILIT) in comparison with SCIT.⁷³ As early as 4 months after the start of therapy, a significant increase in tolerance to nasal provocation in the ILIT-group was found as compared with baseline. By contrast, there were no significant differences in threshold concentrations compared with baseline in the SCIT-treated group at this early stage of therapy. In another DBPC "proof of concept" trial, the same group investigated the effects of transcutaneous immunotherapy using allergen-containing patches, with NPT used as primary outcome parameter.⁷⁴ Actively treated patients revealed significantly decreased scores in nasal provocation tests in the first and second year after treatment compared with baseline. However, the differences in comparison with the group with placebo patches did not reach statistical significance. Taken together, the NPT reproduces the response of the upper airways to natural allergen exposure under controlled and reproducible conditions.⁶⁸ However, this method is a time-consuming and laborious procedure.

As an alternative allergen challenging method, the conjunctival provocation test (CPT) has been performed in several studies as an inclusion criterion and as an outcome measure after pharmacotherapy or SIT.⁷⁵⁻⁷⁷ In a DBPC study of La Rosa and colleagues,⁷⁸ 41 children allergic to *Parietaria*-induced rhinoconjunctivitis were randomized to receive sublingual standardized *Parietaria judaica* extract or placebo. As a secondary outcome parameter, CPT was performed with titrated doses. After 1 year of SLIT treatment the threshold dose in CPT increased in both groups, but to significantly greater extent in the active treatment group. CPT was also investigated

in the Preventive Allergy Treatment (PAT) study, the first prospective long-term follow-up trial addressed to investigate whether SCIT has a preventive effect on the development of asthma.^{43,79} In this trial 147 allergic patients, aged 16 to 25 years, have been analyzed 10 years after initiation of a 3-year course of SCIT with standardized allergen extracts of grass and/or birch or no SCIT (antiallergic medication only), respectively. In addition to a preventive long-term effect on the prevalence of asthma found in this trial, the conjunctival sensitivity in the titrated CPT was significantly reduced in the active group as compared with the control group 7 years after termination of SCIT.⁴³

Riechelmann and colleagues⁸⁰ compared the value of CPT in comparison with NPT on 50 mite-allergic patients, and found a high correlation between both challenge tests with concordant results in 90% of the patients tested. Taken together, the CPT is a very sensitive test for providing information about the patient's clinical allergy status. However, one disadvantage of the CPT is that there is a lack of an objective outcome measure, as positive or negative results are only clinically assessed.

Pollen Chambers

Allergen challenge chambers (ACC) provide a promising tool for evaluating the therapeutic effects of SIT, as this method circumvents some drawbacks of conventional clinical outdoor studies such as the unpredictable levels of pollen, weather conditions during the study period, and high diversity of pollen exposure in different regions.^{5,12,66,81} The exposure chamber facilitates both a controlled and reproducible pollen challenge of allergic patients, as well as the assessment of responses to that challenge, using a predefined protocol.⁸¹ The pollen levels in the ACC can be continuously measured and adjusted to stimulate a similar allergen exposure as in ambient air during the natural pollen season.⁶⁶ Hence this method is capable of reproducing the study conditions of an outdoor allergy study with high specificity and high sensitivity.⁸² Provocation chambers have been widely used to evaluate symptomatic antiallergic medications^{83,84} but have been only marginally employed in allergen immunotherapy.^{85–87} Single challenge tests, however, will not be able to imitate the priming effect seen in real life, which is due to repeated or continuous allergen exposures.

In a DBPC clinical trial on 89 grass-allergic patients, Horak and colleagues⁸⁸ investigated the onset of action of a 5-grass pollen sublingual tablet formulation using the “Vienna” ACC. A standardized allergen challenge with grass pollen and assessment of allergic symptoms (ARTSS) was performed at baseline, week 1, and months 1, 2, and 4 during a preseasonal treatment. SLIT tablets revealed a significant improvement in ARTSS scores versus placebo from the first month of treatment onward.

Taken together, ACCs might be valuable tools in detecting the clinical efficacy of SIT in clinical trials performed independently from the natural pollen season. ACCs have been recommended by both the FDA and the EMA guidelines as outcome parameters in phase 2 trials. However, future studies will be needed to validate ACCs more thoroughly compared to the (natural) seasonal pollen exposure.

Asthma-Specific Outcome Measures

The peak expiratory flow (PEF) and the forced expiratory volume in 1 second (FEV₁) are sufficient, practical, and objective tools for the monitoring of asthma. These tools may vary with individual performance (effort-dependent) and provide only a snapshot of pulmonary function, particularly in subjects with highly variable airway obstruction. Therefore, frequent measurements are necessary to create an appropriate data set reflecting the overall pulmonary function. Furthermore, inhalation tests with metacholine (metacholine bronchial provocation test [MBPT]) are valuable in assessing

nonspecific bronchial hypersensitivity in asthma patients with nearly normal PEF and FEV₁ results despite asthmatic symptoms.

In addition to other outcome variables such as the clinical diagnosis of asthma or CPT results, MBPT has been used in the PAT study described earlier.^{43,79} In contrast to other outcome variables, no significant differences regarding MBPT between the SCIT group and control group were found after 10 years in this long-term observation study.

Sting Challenges as Outcome Parameter After Hymenoptera SIT

The efficacy of Hymenoptera SIT can be estimated by a controlled sting provocation under clinical conditions performed according to international guidelines.⁸⁹ As a predictive value for tolerance to Hymenoptera venom after venom immunotherapy, the sting challenge test is superior to an accidental “field” sting.⁹⁰ However, as challenge tests are high-risk procedures, they should be only performed in specialized centers and not be executed for the diagnosis of patients who have not been treated with SIT. Furthermore, a negative challenge test does not ensure the absence of reactions after subsequent Hymenoptera stings.

IN VITRO PARAMETERS: SURROGATE MARKERS USEFUL TO SOME EXTENT

In vitro parameters such as allergen-specific IgE and specific IgG levels, cytokines such as interferon- γ , interleukins, tryptase, eosinophil cationic protein (ECP), and other inflammatory parameters are valuable additional secondary outcome measures assessing the immunologic effect of SIT in clinical trials (Table 4).⁶ Some examples of these surrogate markers used in SIT trials are characterized below. These markers cannot substitute for the assessment of clinical parameters described earlier, but can be used to assess secondary outcomes in combination with the clinical outcomes or to analyze particular aspects of SIT, for example, immunologic mechanisms of treatment.^{6,10} In its 2008 *Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases* the EMA¹² emphasizes

Table 4 Surrogate markers and paraclinical parameters to evaluate the therapeutic effect of specific immunotherapy	
Organ-Related Provocation	Immunologic Parameters
Skin: skin prick test (SPT), favorably with titrated allergen-doses Intracutaneous test (IT): late cutaneous response	Total IgE, specific IgE and IgG subclasses
Nose and eye: allergen challenge tests, favorably with titrated allergen-doses	Mucosal IgA Mucosal IgE
Lung: spirometric results: FEV ₁ , peak flow variability (PEF), metacholine challenge, allergen challenge	Lymphocyte subgroups Cytokines (eg, IFN γ , IL-5, IL-10, IL-12) Local and systemic inflammatory parameters (eg, adhesion molecules, ECP, tryptase)

Abbreviations: ECP, eosinophilic cationic protein; FEV₁, forced expiratory volume in 1 second; IFN γ , interferon- γ ; IL, interleukin.

Data from Canonica GW, Baena-Cagnani CE, Bousquet J, 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;62:317–24.

that “as long as laboratory parameters such as allergen specific antibodies, T cell reactivity or cytokines are not validated and not correlated to the clinical outcome, they can only provide supportive information.”

Specific IgG Antibodies and Clinical Effects

The relevance of IgG levels for assessing the outcome in SIT remains controversial, as trials have demonstrated variable data in IgG₄ responses.¹⁰ In the first published DBPC trial of SCIT using a cocktail of 5 recombinant grass pollen allergens, IgG₁ concentrations increased approximately 60-fold, peaking during the first 12 months of the study, and IgG₄ levels achieved an approximately 4000-fold increase by the end of treatment, indicating a high allergen-specific IgG antibody response combined with a good clinical efficacy.²⁰ Another 2-year SCIT trial with 2 consecutive preseasonal short-term treatments demonstrated a 48.4% difference in combined SMS after the second year combined with a 210-fold increase of allergen-specific IgG₄.²¹ However, other studies on both SCIT and SLIT have revealed lower increases of IgG₄ responses although they demonstrated clear clinical efficacy.^{33,91,92} Quirino and colleagues⁹³ performed a direct comparison of SCIT and SLIT in adults with grass pollen seasonal AR with and without asthma in a double-dummy design, and demonstrated both procedures to be equally effective in the clinical outcome. The study revealed that only in the SCIT-treated patients clinical efficacy was associated with a significant increase in specific IgG and specific IgG₄. A 2-year study on 29 grass pollen-allergic children also failed to demonstrate changes for specific IgE/specific IgG₄ ratios over time, either in the active group or between active and placebo-treated children.⁹⁴ By contrast, two well-designed studies on SLIT with grass pollen tablets in pediatric patients showed that clinical efficacy was associated with a significant increase of specific IgG₄ levels in grass pollen, although the extent of the increase was lower than in SCIT-studies.^{18,24} Taken together, specific IgG, particularly IgG₄ antibodies, are produced following SIT, indicating an immunologic response to the applied allergen preparation.¹⁰ However, their specific role in the underlying mechanism of tolerance induction as well as the extent and predictive value of the rises of these surrogate markers have to be further evaluated in future trials.

EFFECTS OF SIT ON THE NASAL MUCOSA: CELLULAR LEVEL AND INFLAMMATORY MARKERS

Taking smears or biopsies of allergen challenged mucosal sites of the nose before and after SIT has been used to investigate changes of the inflammatory process at the cellular level in several studies of SIT. In a DBPC study on SLIT in adult patients with rhinitis and asthma monosensitized to birch pollen, a nasal smear for eosinophil count revealed a significant decrease in the active group in the birch pollen season after approximately 18 months of therapy, further aggravated in the subsequent years.⁹⁵ Another study by Durham and colleagues⁹⁶ investigated the effect of grass pollen SCIT on late nasal responses and associated cellular infiltration following nasal provocation by taking nasal biopsies. These tests were performed after a 12-months DBPC trial on SCIT. SCIT was associated with a significantly decreased accumulation of eosinophils and CD4⁺ cells as well as a significant increase in cells expressing mRNA for interferon- γ in the nasal mucosa after provocation.

In an open, randomized SCIT trial conducted by Klimek and colleagues,⁹⁷ the effect of a short-term, preseasonal immunotherapy on ECP and tryptase in nasal secretions of 48 grass pollen-allergic patients was investigated. In both groups of actively treated and placebo-treated patients, ECP and tryptase levels increased significantly during

natural allergen exposure in the grass pollen season. However, the increase in the SCIT group was significantly blunted in comparison with placebo. Taken together, there are interesting experimental findings on the specific effects of SIT on the nasal mucosal level. If and to what extent these tools will be useful in interpreting the outcome of SIT has to be verified.

SUMMARY

The assessment of the clinical efficacy of SIT in clinical trials involves measurement of several clinical parameters. Primary outcome parameters include a measure of symptoms and of the usage of concomitant medication, and should ideally combine both scores in a weighted balance. Both disease-specific (RQLQ) and generic QoL and other surrogate markers are usually regarded as secondary outcome parameters. Taken together, the outcome measures described in this article are valuable parameters in assessing the efficacy of SIT in clinical trials. However, further validation of these parameters in future trials as well as stringent and transparent guidance by the regulators is mandatory.

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