

An evidence-based appraisal of the surrogate markers of efficacy of allergen immunotherapy

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Purpose of review

The assessment of clinical efficacy of immunotherapy in patients with respiratory allergy is highly affected by variability and lack of validated outcome measures such as symptom and medication scores. At the same time, there is a need to identify reliable predictive surrogate markers or biomarkers that may correlate with real clinical endpoints and lead to individually tailored immunotherapy treatment.

Recent findings

In-vivo markers, such as early and late skin reaction as well as immunological parameters such as IgE levels, IgG subclasses, mucosal IgA, lymphocyte subsets, cytokines and local and systemic inflammatory markers, have been proposed as potential surrogate markers.

Summary

Given the poor reproducibility and surrogacy to clinical outcomes shown in various clinical trials, the decreased availability in daily practice and anticipated high costs, an ideal surrogate marker is still not available to allergists.

Keywords

allergen immunotherapy, markers, surrogate

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Introduction

Allergen-specific immunotherapy (SIT) is a well established treatment option for patients with allergic rhinitis with or without associated asthma. Compared with pharmacological therapies, which provide only temporary relief of symptoms, SIT is the only currently available treatment that can modify the allergic disease process. Its clinical efficacy has been shown in many clinical trials and further confirmed in several meta-analyses [1[•],2[•]].

The majority of randomized clinical trials, in order to provide evidence of clinical efficacy of SIT, investigate the effect on symptoms and on the use of rescue medications as 'primary outcome' parameters. However, standardized symptom/medication scores are not currently available and different scoring systems are used in different studies [3].

There is a need for surrogate markers that can objectively measure the response to immunotherapy for use in everyday clinical practice as well as in clinical trials. The National Institutes of Health defines a surrogate endpoint/marker as 'a biomarker intended to substitute for a clinical endpoint'. It is a measure of effect of a treatment

that may correlate with a real clinical endpoint but does not have a guaranteed relationship [4].

It is anticipated that identification of potential surrogate markers will assist in distinguishing responders from nonresponders early on so that immunotherapy can be targeted to those more likely to benefit. Furthermore, surrogate markers may be useful in not only monitoring response and adjusting treatment accordingly but also deciding when to discontinue treatment or predict relapse after discontinuation. The increased knowledge of the underlying mechanisms of initially subcutaneous immunotherapy and more recently sublingual immunotherapy has given rise to novel potential in-vivo and in-vitro markers (given below).

Potential surrogate markers in allergen-specific immunotherapy (adapted from the study by Canonica *et al.* [3]):

- (1) *In vivo*
 - (a) skin prick test reactivity, late cutaneous response.
- (2) *In vitro*
 - (a) immunological parameters,
 - (b) total and allergen-specific ige, igg subclasses, mucosal iga, lymphocyte subsets, cytokines,

- (c) local and systemic inflammatory markers,
- (d) adhesion molecules, urinary leukotrienes, serum eosinophil cationic protein (ECP), FeNO and
- (e) evaluation of quality of life (QoL).

In-vivo markers

A number of physiological parameters have been proposed as biomarkers of an immunological response. A significant reduction of skin reactivity to allergen has been recorded in several controlled studies with sublingual and subcutaneous immunotherapy in both seasonal and perennial allergic rhinitis and/or asthma [5,6]. However, the different biological potency of different diagnostic extracts is a potential drawback in this evaluation [7]. Moreover, even if there is a trend in reduction of early and late skin reactivity in successful immunotherapy in individual patients, this parameter cannot predict the clinical outcome of the treatment.

In patients with seasonal allergy, the assessment of clinical efficacy is further complicated by the variability of pollen seasons. A low pollination can lead to lack of a significant clinical difference, whereas in a heavy pollen season patients may experience increased symptoms despite the treatment being efficacious [8^{••}]. Changes of the specific threshold dose following allergen nasal, bronchial or conjunctival provocation tests can predict a clinical improvement, but a standardized method for such tests is not currently available [9[•]]. Furthermore, a patient is challenged with a high load of allergen in a short time during a provocation test, whereas real life is characterized by a constant low concentration allergen exposure. Thus, even if provocation tests can be valuable tools for determining the onset of efficacy and the optimal dosage of SIT, they are not necessarily appropriate means of predicting clinical symptoms and consequently clinical efficacy. A specific challenge performed before and during or after immunotherapy in a pollen challenge chamber with controlled exposure has been suggested as a reliable method to avoid these difficulties, but in real life only few centres may have access to such chambers in the future [10].

Immunological surrogate markers

Since the earliest immunological discoveries, clinicians tried to evaluate the efficacy of SIT by measuring the reduction of specific IgE levels. However, during successful immunotherapy allergen-specific IgE levels tend to initially increase and later revert to pretreatment levels without levels necessarily decreasing below pretreatment levels [11].

In a recent study [12] of mono-sensitized patients to pollens or house dust mite treated with subcutaneous

Key points

- The availability of surrogate markers of clinical efficacy of allergen immunotherapy might improve the selection and the follow-up of patients who have undergone this treatment.
- Given the poor reproducibility and surrogacy to clinical outcomes shown in various clinical trials, the decreased availability in daily practice and anticipated high costs, an ideal surrogate marker is still not available to allergists.

(76 patients) or sublingual (203 patients) immunotherapy, the serum-specific IgE/total IgE ratio was found to offer an advantage over measuring total IgE or specific IgE separately in predicting clinical response to SIT.

Apart from changes in IgE levels, an increase in allergen-specific IgG has been reported during SIT. These antibodies can block IgE-mediated anaphylaxis *in vivo* and seem to inhibit not only the allergen-induced release of inflammatory mediators from basophils and mast cells, but also IgE-mediated allergen presentation to T cells. Among the SIT-induced IgG antibodies, allergen-specific IgG4 have been suggested to play an important 'protective' role, as they compete with allergen-specific IgE antibodies and can prevent the activation of CD4⁺ T cells, by inhibiting the IgE-mediated antigen presentation [13]. Furthermore, the IgG4 subset which is secreted in significant amounts after prolonged SIT and remains stable over years of SIT treatment, promotes a significant reduction in mast cells and eosinophils, accompanied by a diminished release of inflammatory mediators [14,15]. It has therefore been suggested that increased IgG4 antibody levels correlate with the clinical efficacy of SIT and may be considered as a marker of SIT-induced immune tolerance and decreased allergen sensitization [16].

In a number of studies, subsets of patients with good clinical response, however, did not have significant IgG antibody production, whereas others with increased IgG antibody titres showed no clinical response, raising the possibility that the synthesis of IgG antibodies may reflect high allergen exposure rather than play a vital role in successful immunotherapy [17,18].

The functional assay of serum 'blocking' IgG activity as determined by suppression of FcεRI-dependent basophil histamine release [19] and/or inhibition of FcεRII (CD23)-dependent binding of IgE–allergen complexes to B cells seems to offer a better prediction of the individual clinical response [20]. After 2 years of immunotherapy withdrawal, immunotherapy-induced grass-pollen-specific IgG1 and IgG4 levels have been found to decrease to near preimmunotherapy levels but the

inhibitory bioactivity of allergen-specific IgG antibodies remains unchanged. In this study [21[•]], IgG-dependent inhibitory bioactivity against IgE allergen binding was studied in cellular assay by means of flow cytometry and confocal microscopy to detect binding of IgE–grass pollen allergen complexes to B cells.

An increase in salivary allergen-specific IgA has been reported in a single study [22] after local nasal immunotherapy, without a clear relationship with the clinical outcome. More recently, an increase of serum IgA1 and IgA2 has been demonstrated after sublingual grass immunotherapy [23[•]].

Allergen-specific immunotherapy can modulate different components of the immune system. Cellular modifications consist of a reduction in allergen-induced T-cell proliferation, indicating the induction of peripheral tolerance in allergen-specific T cells and a decrease in antigen-specific Th2-dominated immune response in favour of a Th1 reaction with increased IFN- γ production [24]. The key cell type responsible for coordinating this immunological switch is a heterogeneous T-cell population, called regulatory T cells (Treg). At the cellular level, the crucial factor for successful allergen immunotherapy is the peripheral induction of type 1 Treg cells. Functional studies on type 1 Treg cells, specific in recognizing antigens, revealed that the modulation of Th1 and Th2 responses by type 1 Tregs mostly depends on the secretion of the cytokine IL-10, which has immunosuppressive properties. In fact, IL-10 inhibits the proliferative response of peripheral T cells against specific allergens and plays a central role in the induction of T-cell anergy [25]. *In vitro*, IL-10 enhances the expression of the regulatory factor FoxP3, modulates eosinophil function and reduces pro-inflammatory mediators released by mast cells [17].

Several studies have reported the induction of a population of IL-10 producing T cells by SIT, but it still remains unclear whether this parameter could be used to study the clinical efficacy of SIT [26–30,31[•]].

Another possible marker of clinical efficacy of SIT is the detection of changes in the number or the nature of allergen-specific T cells. On the basis of, for example, Bet v1 tetramer staining studies, the levels and characteristics of circulating birch pollen-specific CD4⁺ T cells can potentially be compared before and after SIT. However, the relevance of this test in the clinical setting is likely to remain limited as Major Histocompatibility Complex (MHC) tetramer reagents are not available for most antigens and they are specific for individual MHC alleles [32].

Recently, transforming growth factor (TGF)- β has also been identified as a key cytokine in successful SIT. Many

actions may account for its relevance, such as the suppression of specific Th1, Th2 and Th17 cells, the induction of FoxP3 and the suppressive function of Tregs. In addition, TGF- β downregulates Fc ϵ RI expression on Langerhans cells and suppresses IgE synthesis [33]. Immunotherapy has also been found to increase the expression of mucosal and circulating T cells producing TGF- β [34–36], and an open study [37] performed on a small number of patients receiving sublingual SIT reported a significant correlation between clinical improvement and increase of serum TGF- β levels. However, the concentration of TGF- β in biological fluids may also be related to the magnitude of allergen exposure [38[•]]. On the contrary, there is evidence that the sub-epithelial fibrosis component of airway remodelling in asthma is mediated by the induction of TGF- β expression, with consequent activation of myofibroblasts which produce extra-cellular matrix proteins such as collagen [33]. Levels of TGF- β are significantly increased in patients with severe asthma characterized by eosinophilic inflammation [39]. At the present level of knowledge, it is still unclear whether TGF- β has to be considered as a friend or a foe.

Local and systemic inflammatory markers

Eosinophils play a crucial role in allergic inflammation and their downregulation has been demonstrated for both sublingual and subcutaneous SIT [40]. A reduction in nasal eosinophils following successful immunotherapy has often been reported, particularly after long-term treatment [41[•]], whereas the effect of SIT on circulatory eosinophils is more debatable [42[•]]. Likewise SIT has been reported to reduce nasal ECP [43], whereas its effect on serum-ECP is less established [44].

Several studies have addressed the effect of SIT on eosinophil activation markers. No change of leukotriene levels has been reported [45], whereas a reduction of exhaled nitric oxide has been reported only during the build-up phase of subcutaneous immunotherapy performed according to a rush schedule [46]. Furthermore, a significant reduction of adhesion molecule Inter-Cellular Adhesion Molecule 1 (ICAM-1) has been reported after successful sublingual immunotherapy [47,48].

A significant problem with evaluating markers of inflammation is that patients having immunotherapy may also be taking at the same time medications such as inhaled steroids which can have an effect on such markers.

Patient-reported outcomes: quality of life

In recent years, increased attention has been paid to the patient's viewpoint about his/her illness and treatment

Table 1 Assessment of quality of life in specific immunotherapy clinical trials

Author	Year	Immunotherapy	Allergen	Instrument
Bousquet <i>et al.</i> [51]	1999	SLIT	GRASS	SF-20
Alvarez-Cuesta <i>et al.</i> [52]	2005	SCIT	MITE	RQLQ
Frew <i>et al.</i> [53]	2006	SCIT	GRASS	RQLQ
Passalacqua <i>et al.</i> [54]	2006	SLIT	MITE	SF-36
Durham <i>et al.</i> [55]	2006	SLIT	GRASS	RQLQ
Moreno-Ancillo <i>et al.</i> [56]	2007	SLIT	OLIVE-GRASS	RQLQ
Powell <i>et al.</i> [57]	2007	SCIT	GRASS	RQLQ
Didier <i>et al.</i> [58]	2007	SLIT	GRASS	RQLQ
Lizaso <i>et al.</i> [59]	2007	SCIT	ALTERNARIA	RQLQ
Colás <i>et al.</i> [60]	2007	SCIT	SALSOLA	RQLQ
Pham-Thi <i>et al.</i> [61]	2007	SLIT	MITE	CAQ
Roder <i>et al.</i> [62]	2007	SLIT	GRASS	Pediatric RQLQ
De Blay <i>et al.</i> [63]	2007	SLIT	GRASS	Mini RQLQ
Dahl <i>et al.</i> [64]	2007	SLIT	GRASS	RQLQ

SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.

options. All these viewpoints, which include preference, adherence, tolerability and QoL, are grouped under the definition of patient-reported outcomes and among them QoL has been the most extensively studied [49]. QoL encompasses the self-perceived effects of an illness and its treatment on the well being. Whereas health status is defined by physicians, usually by means of objective measures, the impact of a disease on QoL is assessed by the patient by means of questionnaires. QoL has rapidly been recognized as a crucial evaluation parameter, and numerous 'instruments' (questionnaires) to measure the impact of respiratory allergy have been developed and validated. Of note, QoL roughly correlates with symptoms and biological/functional parameters, but it considers also many aspects that cannot be measured by standard methods. For this reason, QoL has been suggested as one of the most relevant secondary outcomes in SIT trials [50]. Concerning SIT, an early trial of sublingual immunotherapy clearly showed a significant effect of the treatment on the general well being [51]. Afterwards, some other clinical trials considered QoL (Table 1) [52–64] as a secondary outcome, and all of them confirmed that the effects of SIT on QoL roughly parallel the clinical outcome, that is the reduction in

symptoms and/or medication intake. Looking at the literature, it is clear that QoL assumes a relevant value in the evaluation of the effects of SIT. Nonetheless, it is so far not clear whether QoL can be considered a valid surrogate marker to replace the traditional clinical evaluation, also because the available questionnaires usually explore a previous period of more than 2 weeks, and do not allow a fine daily evaluation.

Conclusion

In conclusion, despite a century of SIT use, many controlled trials providing evidence of clinical efficacy as well as an increased understanding of the underlying immunological mechanisms, clinicians are still looking for biomarkers showing surrogacy to clinical endpoints (Table 2). For widespread use, the ideal surrogate marker should be a low cost tool, efficient and easily available. Nowadays, the study of the early and late skin reaction and level of several immunological parameters such as IgE and IgG antibodies can be easily performed in most allergy laboratories. However, as previously discussed, their predictability of SIT clinical outcomes in the individual patient is poor.

Table 2 Advantages and disadvantages of current potential surrogate markers of specific immunotherapy

	Parameter	Practical use	Predictability of clinical response
<i>In vivo</i>			
	Skin reactivity: early and late reaction	+++	+
<i>In vitro</i>			
Humoral immunity	Total IgE, s-IgE, s-IgA, IgG, IgG1, IgG4	+++	+
	Functional assay of serum blocking IgG activity	++	+++
Cellular immunity	Peripheral cytokine production (e.g. IL-10, TGF-β)	+	+++
	Detection of peripheral allergen specific T cells (tetramer technology)	+	+++
	Functional assay of the ability of T cells to downregulate allergen-specific T response	+	+++
Inflammation	Nasal: eosinophils, ECP, ICAM-1	++	+
	Serum: ECP, circulating eosinophils, s-ICAM 1	+++	+
	Exhaled nitric oxide	+++	+

TGF, transforming growth factor-β.

Furthermore, even if successful, SIT is associated with increased IgG blocking activity; this is solely dependent on the serum levels of IgG antibodies [44]. It appears more clinically relevant to measure the blocking activity of allergen-specific IgG or IgG subsets, particularly IgG4 and IgG1 rather the crude levels in serum [65]. The ratio of serum-specific IgE to total IgE seems to be more promising [12] but it has to be confirmed in larger populations and especially in poly-sensitized patients encountered in clinical practice [66].

Functional assays seem to offer a better predictability but their reproducibility and cost need to be taken into account. The study of immunological cellular responses has led to a deeper understanding of the mechanisms of immunotherapy. However, in the complex network of cells and cytokines involved in the immune response, a sole key cytokine related to a positive outcome of SIT has not yet been identified. Assays of T-cell tolerance (e.g. peripheral IL-10 production, identification of allergen-specific T cells by tetramer technology, functional T-cell assays) are currently under development. The potential of clinical markers of tolerance to predict individual response to immunotherapy needs to be critically evaluated. These novel assays will require evaluation in large well designed controlled trials in order to determine whether they may predict when to start, when to discontinue SIT or predict relapses and, consequently, the need for a further course of immunotherapy [25].

Health-related QoL assessment has been shown to be a reliable evaluation parameter, which is currently recommended as a relevant secondary outcome. Unfortunately, QoL evaluation has no predictive value for clinical efficacy.

For all these reasons, the ideal surrogate marker is still eluding allergists. However, there is hope that with the continued efforts of researchers in understanding the mechanisms of immunotherapy, this can become a reality in the near future.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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