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Predictive biomarkers of clinical efficacy of allergen-specific immunotherapy: how to proceed

“Although we are able to distinguish between actively and placebo-treated patients, candidate biomarkers of effect or biomarker combinations remain to be determined.”

KEYWORDS: biomarkers of effect ■ blocking antibodies ■ dendritic cell phenotype ■ immunotherapy ■ mode of action ■ standardized clinical end point

Various mode-of-action studies have been conducted to describe and substantiate the immunologic mechanisms behind the long-lasting effect of allergen-specific immunotherapy (SIT) and how it changes the course of IgE-mediated allergic disease. These randomized, double-blind, placebo-controlled studies have reported both cellular and humoral changes systemically and in the target organ following SIT. However, demonstrating that these immunological changes can be used to monitor the effect of treatment has proven challenging. Although we are able to distinguish between actively and placebo-treated patients, candidate biomarkers of effect or biomarker combinations remain to be determined. The validation of such biomarkers may need to involve unconventional ways to evaluate clinical effect, such as challenge chambers or controlled provocation tests of individual organs, to clearly distinguish between strong and weak or early and late responders, as discussed below.

Clinical effect of SIT

Specific treatment of allergic diseases has been based mainly on empirically established traditions until small-scale clinical studies delivered some evidence of the beneficial effect of subcutaneous administration of allergen extracts, what we now call allergen SIT. During the second half of the 20th century, double-blind, placebo-controlled trials, pioneered by Lowell and Franklin, provided supporting evidence that allergen immunotherapy results in reductions of symptoms and the need for rescue medication, reduced skin responses and increased allergen threshold levels in provocation tests [1,2]. The clinical effect has recently been confirmed in several meta-analyses for both subcutaneous [3] and sublingual immunotherapy [4]. Furthermore, SIT

has been demonstrated to modulate the course of the disease, inducing a lasting effect after the withdrawal of treatment [5–8], inhibition of the progression from rhinitis to asthma [9,10] and even a reduced risk of new sensitizations [11]. This lasting imprint on the course of the allergic disease clearly indicates that immunological changes in the B- or T-cell memory compartment are involved in symptom relief.

Candidate biomarkers

In diseases such as stroke and coronary artery disease, a direct link has been established between the drug in question and a measurable biological effect (blood pressure and cholesterol levels, respectively), leading to symptom relief [12]. In asthma, periostin and eosinophil counts have been used in the development of anti-IL-13 and -IL-5, respectively, for treatment of distinct asthma subtypes [13]. For SIT, various mechanistic models have been proposed during the last two decades. The main immunological effects have now been narrowed down to the induction of blocking antibodies and a shift in Th1/Th2 balance and induction of Tregs [14–16]. However, changes in cell numbers or activation levels for eosinophils, mast cells and basophils, as well as enhanced IL-12 production by APCs, have also been described [14], and all of these effects may contribute differently to the symptom relief in the individual patient. This variety of immunological and inflammatory changes observed during immunotherapy could suggest that a direct link between an individual immunological measurement and the clinical effect of immunotherapy will be difficult to establish. Moreover, the variability of the currently accepted clinical end points will add to this complexity, so highly standardized surrogate end points for clinical effect have to be established.



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Associations have been found between clinical responses to grass pollen immunotherapy and clinical surrogates such as skin prick test titration to allergens, allergen-specific bronchial responsiveness and nasal or conjunctival challenges [17,18]. However, true correlations with the magnitude of the clinical response in terms of reduced symptoms or improved quality of life have yet to be convincingly demonstrated. We have recently standardized a nasal allergen challenge procedure for grass pollen allergy [19], and this method is currently used to relate nasal challenge symptoms to immunological changes during both sublingual and subcutaneous grass pollen immunotherapy.

Examples of biomarker studies

A consistent finding related to SIT treatment is an increase in allergen-specific non-IgE antibodies [20] and several investigations have demonstrated that serum antibodies have the capacity to reduce *in vitro* reactions mimicking allergic responses, such as IgE binding to allergen, IgE-facilitated antigen presentation and basophil activation [7,21,22]. We recently followed-up on the link between changes in symptom and medication scores [23] and the time- and dose-dependent increases of IgG4 titers, IgE-blocking factor, or facilitated allergen binding (FAB) inhibition (serum inhibitory activity for binding of allergen-IgE complexes on to B cells) in a double-blinded, placebo-controlled study of grass pollen immunotherapy [24]. There was a modest but significant inverse correlation between serum inhibitory activity (IgE blocking and FAB inhibition) identified immediately after up dosing (at the first maintenance injection) and subsequent combined symptom and medication scores during the pollen season. These findings suggest that biological inhibitory activity of postimmunotherapy sera reflect the mechanism of efficacy (either directly or indirectly) and strengthen the value of IgE-FAB inhibition and IgE-blocking factor as biomarkers of the clinical response to immunotherapy.

However, the lack of baseline clinical data during the preceding pollen season makes it impossible to compute a change from baseline of combined symptom and medication scores for comparison with changes in serum inhibitory activity, and this may partly explain that only modest correlations have been reported.

By contrast, changes in clinical end points during sublingual grass allergen immunotherapy were obtained in a recent clinical trial conducted in an experimental exposure chamber (EEC).

The main finding reported by Zimmer and colleagues [25] was that the expression of signature genes related to tolerogenic dendritic cells (DCs) were changed during sublingual immunotherapy and that the quantitative change in gene expression in unstimulated peripheral blood mononuclear cells correlated with the change in symptom and medication scores observed after allergen exposure under the controlled conditions in the EEC. The authors used the average clinical response in the placebo group to distinguish between responders and nonresponders in the actively treated group and found that modest (R-values between 0.29 and 0.41), but significant correlations could be demonstrated in the responder group, while no such correlation was observed among the nonresponders. Changes in antibody titers or antibody-mediated effects were not investigated by Zimmer *et al.*, but they report that changes in Treg numbers or the balance between different T-cell subsets were not found, even though this could have substantiated the importance of the changed expression of genes linked to tolerogenic DCs.

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It would be surprising if a single biologic variable were to correlate absolutely with the clinical expression of symptoms. Even during a controlled allergen exposure, the cytokine milieu relevant for local T-cell activation, influence of T-cell priming, variability in basophil, mast cell and eosinophil numbers, and reactivity may not be reflected in changes in either DC- or antibody-mediated readouts. For these reasons it is unlikely that a single immunological change would correlate precisely with symptoms following immunotherapy, so the observed moderate but highly significant correlations may be of major importance.

General strategy to validate biomarkers

Putting these obvious obstacles aside, it is of key interest to know if immunological changes truly do play a causal role in SIT. Objectives include understanding the mechanism of treatment efficacy, early prediction of treatment response or nonresponse, surrogate end points, utilization in clinical drug development and so on. This

motivates the aim to assess and quantify if the observed treatment effect is mediated by, for example, IgE-blocking components. In mouse models the use of knockout mice or depletion experiments would reveal the true effect of a biomarker in question, but we have to rely on properly designed trials and complex statistical analyses to evaluate the probability of causal relationships between immunological and clinical outcomes.

The methodology to assess and quantify whether an observed treatment effect is fully (surrogate end point) or partially mediated has been discussed in the statistical literature [26]. According to this, a surrogate end point requires fulfillment of the following four steps:

- Treatment affects the surrogate end point;
- Treatment affects the clinical end point;
- Surrogate end point affects clinical end point;
- The effect of treatment on the clinical end point is mediated by the surrogate.

The relationships between the treatment and end points can be modeled through linear regressions and the fulfillment of all four steps provides supportive evidence for a surrogate end point (fully mediated) or a partially mediated effect. To fulfill step four, statistical nonsignificance of the treatment effect is required so that treatment can be removed from the model and the effect on the clinical end point is fully described by the surrogate. However, because there may be other explanations for a statistically insignificant treatment effect, than 'no treatment effect' the fulfillment of all four steps can only ensure necessary conditions for surrogacy, but cannot ensure sufficient conditions. In addition, a perfect correlation (perfect step three) between the clinical outcome and proposed surrogate end point or mediating variable is not enough evidence for validation in itself. Even perfect correlation does not imply validity of a surrogate end point [27].

There is an increasing body of literature from different areas on the mathematical concepts of causal inference and causal mediation analysis [28,29]. One outcome from this literature is the formal mathematical description of the required assumptions for which it is possible to identify a causal-mediated effect. However, these assumptions are nonrefutable and cannot be directly tested from observed data. Therefore, Imai *et al.* provided a method to perform sensitivity analysis of these nonrefutable assumptions required for the indirect-mediated effect to be causal [28]. Two assumptions would be required. The first is

fulfilled if treatment is randomized in the trial. The second assumes conditional independence of the clinical end point and mediator given the observed treatment and pretreatment confounders. This corresponds to assuming that, among those patients who are in the same treatment group and share the same pretreatment characteristics, the mediator can be regarded as if it was randomized.

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A measure to quantify the degree of mediation is Freedman's proportion of treatment effect explained (PTE). The validity of the PTE depends crucially on the absence of an interaction between the effect of treatment and the proposed surrogate end point in step four. Such an interaction would suggest that the effect of treatment on the clinical end point was not fully acting through the surrogate. Although this estimate suffers various limitations, it may be useful to compare two potential surrogate end points [26].

With the subgroup (100K and placebo, and available blood samples) from Frew *et al.*, all of the four points above are fulfilled in case of Δ -FAP, as well as the Δ -IgE-blocking factor [23]. The estimated PTE is 40% for δ -FAP and 33% for IgE-blocking factor, suggesting that these immunological changes account for 30–40% of the treatment effect. Similar modeling may be performed with the data from Zimmer *et al.* [25] and hopefully future immunotherapy studies will be performed out of season and include nasal challenge or EEC data. This should make it possible to determine change from baseline clinical effects and leave out the medication use confounding all in-season evaluations of the clinical effect of immunotherapy.

Taken together, the complexity of the clinical manifestations, the various immunological effects theoretically involved in these clinical changes, the variability and specificity of immunological analysis applied and, possibly most important, the high degree of variation in the evaluation of clinical effects, makes it extremely difficult to establish correlations. However, future trials with highly standardized clinical end points and insight into new immunological mechanisms may provide data to evaluate

PTE for individual immunological changes or a combination of these to move towards establishing true surrogate end points for immediate and long-term effects of SIT.

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