

In vivo and in vitro allergy diagnostics: it's time to reappraise the costs

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Abstract

Background: The in vivo skin prick test (SPT) is widely considered less expensive than in vitro γ -immunoglobulin E (IgE) determination in the diagnosis of allergy. The aim of the present paper is to evaluate the relevance of component-resolved in vitro diagnosis in comparison to extract-based diagnosis and the relative global costs in relation to clinical outcomes.

Methods: For 50 individuals with suspected seasonal allergic rhinitis, we compared the costs of skin testing with those of specific IgE antibody measurement.

Results: The costs were higher for in vitro than in vivo testing. However, the clinical information obtained using recombinant reagents allowed correct identification of the sensitizing molecule.

Conclusions: Recombinant allergens for specific IgE in vitro measurement provide more reliable information for immunotherapy prescription. This should be translated into a significant reduction in the overall costs sustained by the healthcare system.

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Keywords: allergen-specific immunotherapy; cost comparison; IgE determination; recombinant allergen; skin prick tests.

Introduction

Allergy diagnosis is based on accurate clinical history and subsequent performance of in vivo skin prick tests (SPT) or on in vitro tests [specific immunoglobulin E (IgE) determination].

In view of its simplicity, higher sensitivity, rapid interpretation, and low cost, skin testing is widely considered preferable to specific IgE determination (1). However, in recent decades improvements have been made for in vitro measurements in terms of performance characteristics and response time thanks to the introduction of more reliable and completely automated methods that provide results within a few hours (2). A growing body of evidence demonstrates that the diagnostic sensitivity and specificity of in vitro measurements are similar, if not superior, to

those of in vivo skin testing (3–8). To the best of our knowledge, there is no evidence in the literature demonstrating that skin testing is less costly than IgE measurements, although the difference in direct costs is evident. The aim of the present study was therefore to compare the real costs of in vivo and in vitro tests, and the suitability of the overall clinical information provided by the two different tests in translation into specific and different therapeutic options. We developed a model for calculating the direct and indirect costs of the two techniques and their overall costs incurred in the management of patients, which is based on the information obtained by SPT vs. in vitro IgE measurement. The model was used for patients with inhalatory allergy to grass pollens.

Materials and methods

Theoretical model

We evaluated the direct costs of in vivo (SPT) and in vitro (specific IgE measurement) techniques and ascertained the costs of patient management based on the different information provided by the two diagnostic approaches, especially in terms of immunotherapy. In particular, using in vitro recombinant allergens, a patient's individual IgE reactivity profile can be quantitatively established. The presence of IgE to cross-reactive allergen components can thus be determined and used to predict clinically relevant sensitization to allergen sources that contain immunologically related allergens. Component-resolved diagnosis thus allows accurate identification of molecules for specific immunotherapy to which a patient is sensitized. In vitro diagnostics with recombinant allergens, in fact, indicates whether a patient is sensitized to minor or major allergens, thus obviating cross-reactivity-induced diagnostic problems (9–13) by identifying the allergenic molecule capable of inducing sensitization and defining the ideal candidates for specific immunotherapy (SIT). In particular, inhalatory allergies due to grass allergens are considered the paradigm for this clinical approach (14–16). When IgE antibodies against the major allergens of grass are present in conjunction with symptoms during the grass pollen season, SIT with grass pollen extract is indicated. However, if no proof of genuine grass pollen sensitization can be found (i.e., patients sensitized not only to Php1, but also to Php7 and Php 12), grass pollen immunotherapy is contraindicated because of the lack of standardization of the extract with respect to calcium-binding protein or profilin. The traditional immunotherapeutic approach, in fact, has several drawbacks related to the quality of the extracts used and the risk of inducing anaphylactic reactions (12, 13, 17–19).

Patients

The costs of in vivo and in vitro allergy diagnostics were evaluated for 50 consecutive patients (33 females, 17 males; age 12–56 years, mean 32 years) referred to our specialized outpatient unit with suspected seasonal allergic rhinitis.

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Table 1 Costs for in vivo skin prick and in vivo IgE testing.

Skin prick testing		In vitro testing	
Extract drop €0.50 × 10	€6.00	Blood collection	€2.00
Prick lancet €0.10 × 10		Testing (including reagents and materials for 12 allergens)	€49.25
Physician's time for skin testing (8 min)			
Test interpretation (4 min)		Result validation	
Physician charge (€38.00/h)	€17.00	(physician charge €38.00/h)	€1.00
Total amount	€23.00	Total amount (including technologist costs)	€52.25

To investigate in vivo testing (SPT), we evaluated the costs of the allergenic extract, the sterile prick lancet, the physician cost per hour, and the time taken to conduct the test. For in vitro testing (IgE testing) we evaluated the costs of: 1) each test; 2) the laboratory technician cost per hour; and 3) blood collection.

The costs incurred in taking the patient's medical history and making an objective examination for in vivo and in vitro diagnostics were considered equivalent.

In vivo analysis

Ten major allergens (*Alternaria*, *Dermatophagoides pteronissimus*, *D. farinae*, dog and cat epithelium, Graminaceae mix, Compositae mix, *Parietaria*, Betulaceae and olive tree) and positive and negative control solutions were assayed. The test outcome was interpreted after 15 min by tracing and assessing the average wheal diameter and the erythema area. The results were expressed using plus signs to reflect the degree of the response according to the guidelines of the Italian Society of Allergology and Clinical Immunology (20).

In vitro analysis

Specific IgE measurement was performed using a fully automated system (UNICAP 1000, Sweden Diagnostics, Uppsala, Sweden) for the following allergens: Php 1 (Graminaceae major recombinant allergen), bet v 1 (Betulaceae recombinant major allergen), rPhp12 (profilin recombinant), rPhp7 (calcium-binding protein), w211 (recombinant *Parietaria*), t9

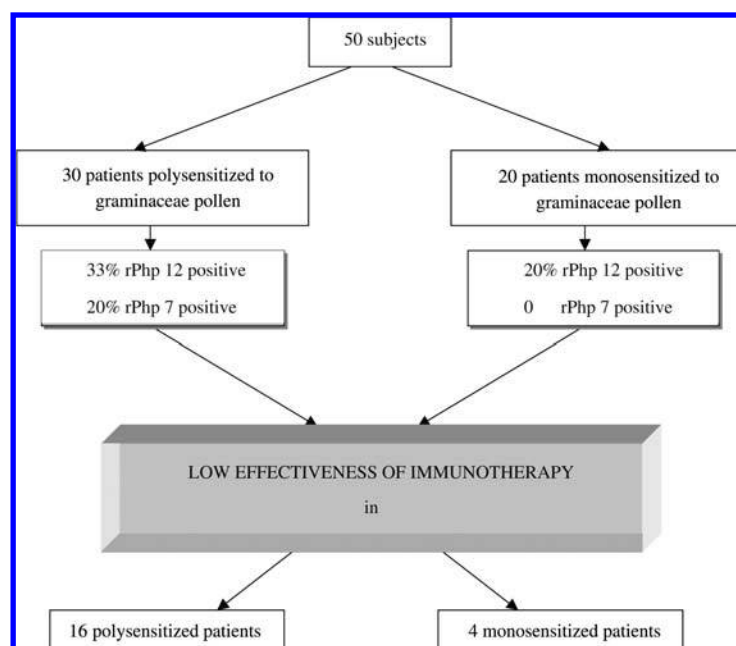
(olive tree), w5 (*Artemisia*), d1 (*D. pteronissimus*), d2 (*D. farinae*), m6 (*Alternaria*), e1 (cat dander), and e5 (dog dander).

Results

The costs of in vivo and in vitro testing are shown in Table 1. Reagents and devices used for skin prick testing in an individual with suspected allergy cost €0.60 (€0.50 for the extract drop, and €0.10 for the prick lancet for each extract, with 10 extracts tested). The physician took 8 min to perform the skin tests, 15 min to wait for the skin reaction and 4 min to read and interpret the skin reaction. Altogether, as the physician charged €38.00/h, the cost of the allergy test amounted to €23 for each patient.

The in vitro measurement expenses were calculated by considering €4.00 as the cost of each determination (€48.00 for measuring 12 allergens) and €1.25 as the fee of the laboratory technician, who takes less than 3 min to perform the test for each patient using fully automated procedures. Including blood collection (€2.00) and result validation (€1.00), the total cost of in vitro testing for a single patient was €52.25.

Of the 50 patients investigated, 30 (Figure 1) were polysensitized (not only to Php 1 or Php 5, but also to Php 7 and Php 12), predominantly showing positivity

**Figure 1** Characteristics of subjects involved in the study.

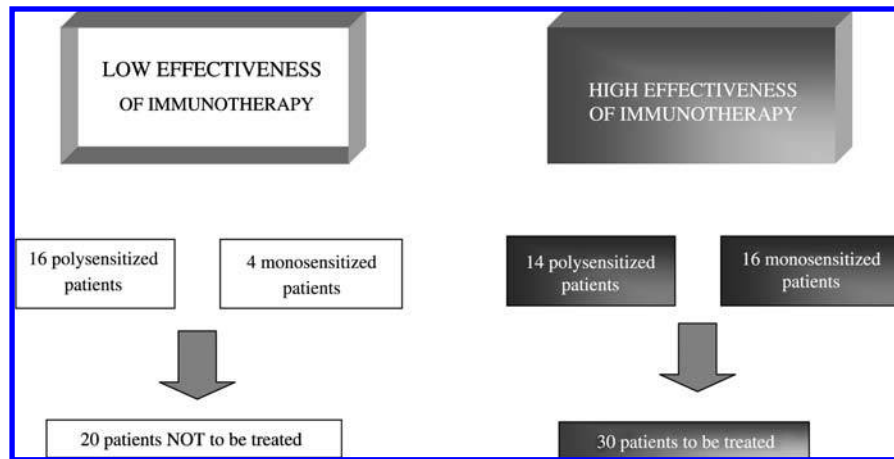


Figure 2 Clinical information provided by recombinant allergens.

to Gramineae pollen, and 20 were monosensitized to grass pollen. In the former group, minor allergens such as calcium-binding protein (rPhp 7) were positive in 20% of the subjects and panallergens such as profilin (rPhp 12) were positive in 33% of the subjects. In contrast, in the monosensitized patients, profilin was positive in 20%, whereas rPhp 7 showed a negative response.

Defining the allergenic determinants to which a patient is sensitized is a crucial factor suggesting or contraindicating SIT, which is only an effective treatment for allergic rhinitis if adequate doses of standardized allergens are administered, often providing long-term benefits that persist after treatment completion (16). The minor allergens (Phl 7 and Phl 12) are typically not used for standardization of extracts. Therefore, these allergens might effectively be under-represented in the therapy extracts, leading to non-effective therapy or even to strong side-effects by

sensitizing patients to the major allergen. As immunotherapy extracts are only standardized for some allergenic determinants (rPhp1–rPhp5), the utilization of recombinant allergens predicts that immunotherapy efficacy will be poor in 16 (53%) polysensitized and four (20%) monosensitized patients. Therefore, in 20 of the 50 subjects studied, immunotherapy would be contraindicated (Figure 2).

Data from the literature demonstrate that patients who underwent SIT, even if sensitized to allergens other than those present in the extracts used, not only had no expected advantages (17–19), such as a reduction in the severity of symptoms of seasonal allergic rhinitis, improvement in rhinoconjunctivitis-specific quality of life or amelioration of the natural history of the allergic respiratory disease present, but they also required concomitant medication (antihistamines for rhinitis, inhaled corticosteroids for asthma). Therefore, the direct cost of 3 years of immunotherapy

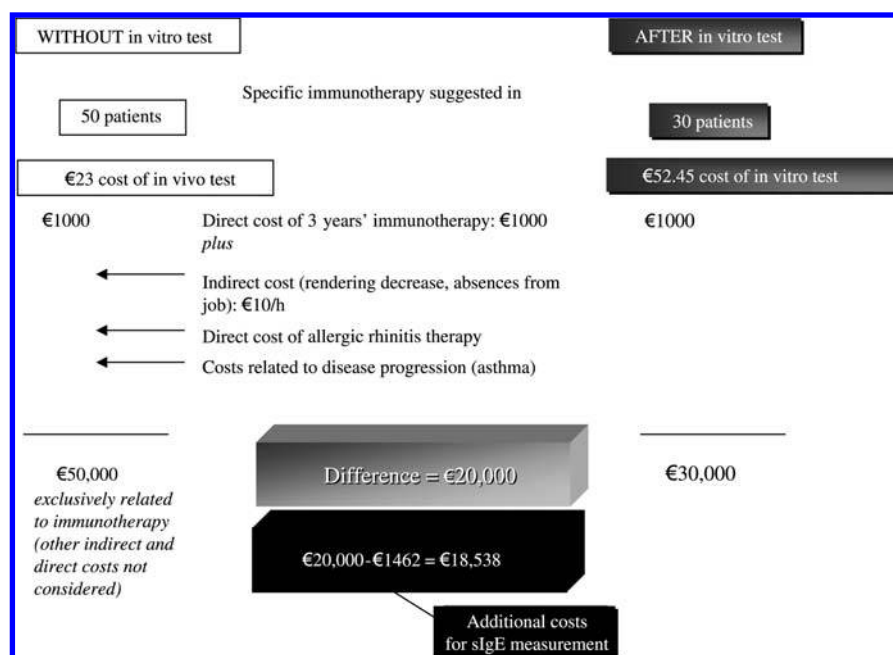


Figure 3 Patients treated with specific immunotherapy without and/or after in vitro testing.

(€1000) is burdened by other indirect and direct charges not included in the present study.

Therefore, we calculated the different costs of patient management based on the information provided by in vivo and in vitro testing (Figure 3 left and right, respectively). The immunotherapeutic approach based on in vivo skin testing (€1150 for in vivo testing of 50 patients) demonstrates low effectiveness if there is no proof of genuine grass pollen sensitization. Moreover, 16 polysensitized and four monosensitized patients could waste their money without achieving the expected advantage, adding to the direct cost of immunotherapy such indirect costs as those for rendering decrease, absence from job, allergic rhinitis therapy and disease progression.

Active immunization with standardized allergen extracts (€2612 for in vitro testing of 50 patients), which is possible for known molecular spectrum sensitization (Figure 3, right), is highly effective. Therefore, the cost is exactly equivalent to the direct cost of 3 years of immunotherapy (€1000).

Discussion

Agreement between in vitro and in vivo testing in the etiological diagnosis of allergic diseases is reported to be greater than 90% (21). In view of the lower costs of skin testing, specific IgE measurement is currently recommended only for patients who cannot suspend drug therapy for their skin condition, those at high risk of anaphylaxis, and those with pathological skin conditions (e.g., severe dermatitis, dermatographism) precluding testing; it is also occasionally performed in patients refusing to undergo skin tests (22). The crucial question is whether or not the additional cost of performing in vitro determination of IgE is decisive in determining the criterion used for treatment strategy. We must highlight that diagnosis using allergen extracts can help to identify sensitization to a particular allergen source, but will not provide information on the molecular identity of the disease-eliciting allergen. In contrast, using recombinant allergens it is possible to define allergen molecules rather than allergen extracts (mixtures of allergens and other non-allergenic components). Therefore, our detailed analysis of costs showed that, overall, skin testing is two-fold less expensive than IgE measurement (€23 vs. €52.25). However, it is important to definitively establish whether the quality of the information provided using this tool is as good as that provided in vitro using IgE testing.

In vitro testing costs include intra- and inter-assay quality controls (2–8), which are not conducted for skin testing, with any assessment of clinical efficiency left to the specialist. As no quality control is conducted for in vivo tests, they cannot easily be compared among different centers. Besides, the use of recombinant allergens, currently available only for in vitro diagnostics, allows identification of the sensitizing molecule in each patient. Patients can thus be defined as allergic to a major or minor determinant, to a

cross-reactive protein with other families; above all, it can be established whether they are sensitive to the allergens present in the extracts used in SIT. Consequently, the information obtained through in vitro testing is more valuable. SPT, now performed with the allergenic extract, can therefore be used as a first-line test. However, if SIT is to be prescribed, an in vitro test is always required for analysis of recombinant epitopes (23, 24). While in vitro testing using recombinant antibodies is not the only means of investigating sensitization for individual allergen molecules, safety issues and practicality make this investigation difficult in vivo. In addition, because of the proven importance of cross-reactive carbohydrate determinants (CCDs), highly purified native molecules or recombinant allergens produced in plant expression systems that are able to generate specific N-glycans should be preferred over the use of recombinant allergens produced in *E. coli* (25). In view of the efficacy of SIT, in vitro testing provides a sensitization spectrum, thus enabling specialists to differentiate between candidates for treatment and patients for whom treatment is contraindicated, with a consequent optimization of patient management and an improvement in the cost/benefit ratio. The findings reported in the present study demonstrate that in vitro testing shows that immunotherapy effectiveness is poor in 40% of patients, thus allowing the identification of patients who should not be treated, i.e., mono- or polysensitized patients against allergenic components not included in the vaccine formulation.

If the sensitization spectrum is not analyzed, the immunotherapy outcome may be compromised, with an enormous increase in costs (Figure 3). In particular, in Italy the financial cost of extracts for 3-year immunotherapy by injection is in the region of €1000. There are also costs not considered in our evaluation: indirect costs (reduced quality of life, compromised productivity at work or school) and costs related to disease progression caused by ineffective hyposensitizing immunotherapy. The model presented has been validated in a limited group of patients. The true validity of the model, however, should be assessed in a more representative number of patients. Moreover, the model presented shows that the avoidance of in vitro testing significantly affects total costs, which increase to €50,000, while these are €30,000 for patients identified as ideal candidates by in vitro testing using recombinant allergens. Considering the additional costs derived from in vitro testing (€52.25 vs. €23.00), the total additional costs for laboratory testing for 50 patients amount to €1462. The difference in costs, therefore, suggests that the addition of laboratory tests leads to a saving of €18,538 in the management of these patients. In our experience, true economy in terms of the cost/effectiveness ratio is achieved not only by reducing the cost per test or by requesting the cheaper test, but also by improving the appropriateness of diagnostic testing. The difference in costs (€29.25) is, in fact, largely offset by a saving of at least €18,538. Therefore, adequate management of patients with seasonal allergic rhinitis hinges on

appropriate use of the tools available to identify candidates suitable for immunotherapy.

The present investigation has several drawbacks: a) costs were calculated taking into consideration the type of reimbursement used in the Italian healthcare system; b) the theoretical model was applied to a limited number of patients with inhalatory allergies treated in a single center; and c) the clinical efficacy stated for allergen-specific immunotherapy in polysensitized patients was derived from literature data (26). While points a) and b) might indicate limitations of this investigation, suggesting that further studies are required, the poor efficacy of hyposensitizing vaccine therapy in polysensitized patients cannot be resolved by conducting randomized blind studies because of the risks associated with this type of therapy. Despite these limitations, available evidence on comparing the costs of skin testing and in vitro testing with recombinant allergens highlights the substantial differences in the quality of clinical information obtained using the two different types of test. In vitro IgE determination is more costly, but it can lead to an important improvement in patient outcome, and is therefore of crucial importance in choosing a suitable therapeutic strategy. Therefore, the choice of appropriate test is at the core of high quality care, but it is also a cornerstone in containing the financial burden of healthcare.

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