

Role of in vitro testing in food allergy

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Abstract

Oral food challenges remain the gold standard for the diagnosis of food allergy. Nevertheless, the allergy workup is based on the presence of a clinical history, which is evocative of an immune-allergic reaction, and the first assessment is usually the performance of skin prick tests. Based on these results, allergists are used to evaluate the presence of serum-specific IgE, which are today the most commonly prescribed in vitro test for the evaluation of a possible food allergy. Other in vitro tests include the basophil activation test, that is becoming more and more employed by clinicians and not only by researchers, and the evaluation of serum IgG4, which is still an issue of debate in the allergy community. The present paper reviews the use of these in vitro tests for the diagnosis of food allergy.

KEYWORDS

basophil activation test, component resolved diagnostics, food allergy, IgE-mediated, IgG, specific IgE

1 | INTRODUCTION

IgE-mediated food allergy (FA) may be diagnosed based on the combination of a suggestive clinical history and positive in vivo and in vitro allergy testing.¹ In vitro diagnostic workup, in clinical practice, mainly includes the assessment of allergen-specific serum IgE antibodies. However, specific IgE tests cannot distinguish between sensitization and allergy. Therefore, an elimination diet should be advised, until the oral food challenge (OFC) may be performed. The basophil activation test (BAT) is a functional assay and measures

basophil response to allergen cross-linking IgE. It has the potential of increasing the specificity of the in vitro tests, to possibly avoid potentially harmful OFC.² As for IgG, there is scarce information on their role in allergic diseases. IgG is commonly known as the "blocking antibodies," as they can inhibit serum IgE and "block" their pathologic responses. Specific IgG testing is unconventional tests that have been evaluated for a few years to assess their role in the FA diagnostic workup.³

In the present paper, we would like to highlight the utility of these tests in clinical practice.

The authors Arasi, Barni and Mastroianni equally contributed to the present paper and should all be considered as first authors.

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2 | SPECIFIC IGE

In vitro tests to measure serum levels of allergen-specific IgE (sIgE) have been used for many years and represent a milestone in FA diagnostics. Several automated and standardized systems are currently available and require a small amount of serum (generally in the order of microliters), not necessarily fresh-collected (if samples are properly stored). According to the World Health Organization Reference Standard, sIgE is quantified in kilounits per liter (kU/L) with 1 unit equaling 2.42 ng of IgE⁴; however, levels determined with different methodologies may not be comparable.

Allergists have been interested in sIgE levels as a potential reliable marker to predict the severity of food-induced allergies. Cutoffs for sIgE have been highly investigated in the past. However, the initial enthusiasm has progressively faded since they have shown high sensitivity but poor specificity to diagnose FA. There is a wide variability among studies, dependent on many items, including the specific prevalence of the disease and co-morbidities into the respective study population, criteria for referring to oral food challenge (OFC), OFC protocols, and criteria for OFC outcomes.⁵ Therefore, no general cutoff levels are recommended.

Conventional IgE testing uses natural extracts containing a complex mixture of proteins. IgE testing for specific molecular allergens allows a more precise profiling of the allergen-sIgE repertoire and is available for single allergens (ELISA) and for multiple allergens (microarrays). Molecular allergology is not always necessary in FA diagnostics but it may improve the diagnostic workup and the management of FA in some circumstances.⁶ For instance, it may be useful in case of sensitization due to cross-reactivity (eg, birch pollen allergic patients tested for hazelnut or peanut allergy, because of sensitization to the so-called panallergens which have a homologous molecular structure) and may add value in predicting the severity of clinical pattern (eg, among patient allergic to tree nuts, the sensitization to stable allergens such as lipid transfer proteins and seed storage proteins is usually associated with more severe symptoms, whereas profilins and PR-10-like proteins are usually responsible for milder symptoms such as oral allergy syndrome). Furthermore, IgE against sequential casein and ovomucoid epitopes is a marker of persistent milk and egg allergy, respectively, and they may help predict possible tolerability to extensively heated food.

3 | BASOPHIL ACTIVATION TEST (BAT)

The basophil activation test (BAT) is an in vitro test that uses flow cytometry to assess the percentage of activated marked basophils, upregulated after the bounding of IgE antibodies to their high-affinity IgE receptor.² It may be used not only with the allergen extract, but also with single allergen components.⁷ As for cow's milk allergy, the BAT showed a significantly higher diagnostic accuracy than SPT and specific IgE, with higher specificity (86%), sensitivity

Key messages

In vitro tests are very useful to complete the allergy workup in food allergic patients. sIgE allows to assess the allergen patients are sensitized to and to evaluate both the severity of the allergy and the possible existence of cross-reactions. The basophil activation test has an elevated specificity in predicting food allergy and should be implemented in clinical practice. IgG₄ testing should not be used as a diagnostic tool in food allergy.

(88%), positive predictive value (81%), and negative predictive value (96%).⁸ Even considering peanut allergy, the BAT showed high specificity (75%-100%) and high sensitivity (77%-98%).² The higher specificity of the BAT was confirmed in studies on eggs and tree nuts allergy as well.^{2,8}

The BAT may also be used to assess the severity of the allergic disease: For example, in those patients reacting to very small amounts of food proteins, it shows basophil activation even at lower concentrations; also, in case of patients at risk of a more severe reaction, the percentage of activated basophils is greater than in other patients.²

Requiring a small volume of blood (about 1 mL) and being minimally invasive, BAT could be well accepted by patients and families.⁷ However, the common system used for such test requires fresh blood, and the main limit for performing the BAT is therefore the presence of an equipped laboratory close to the clinic.

Recently, the mast cell activation test has been evaluated for FA as well, showing a superior diagnostic accuracy compared with other tests, but further studies are needed to fully evaluate this procedure.⁹

4 | IGG₄

The determination of serum IgG₄ (sIgG₄) is not recommended as a tool in the diagnosis of FA, as clearly expressed by the International Consensus ON (ICON) document on food allergy that was outlined under the guidance of the European Academy of Allergy and Clinical Immunology (EAACI) as well as the American Academy of Allergy, Asthma and Immunology (AAAAI) and the World Allergy Organization (WAO).¹ It has been shown that the presence of sIgG₄ against foods reveals a physiological response of the immune system to the repeated exposure to food antigens and, for such reason, sIgG₄ should not be considered as a sign of a hypersensitivity reaction but rather as a marker of immune tolerance associated with the activity of regulatory T cells (Treg). In a study,³ Stapel et al determined sIgG₄ against commonly consumed foods in 13 healthy volunteers. All patients had positive sIgG₄ without presenting clinical symptoms after intake assumption of the tested

food. Therefore, a positive IgG₄ value did not correlate with the FA but more likely with a prolonged exposure to food antigens, their presence being therefore more beneficial rather than harmful for patients. Moreover, a high level of cow's milk-sIgG₄ antibodies was observed in cow's milk allergic children who acquired tolerance.¹⁰ In another study¹¹ performed on a group of ovalbumin-allergic children, a group of ovalbumin-tolerant ones, and a control group, no statistical difference of ovalbumin-specific sIgG₄ was found between the three groups.

5 | CONCLUSIONS

The diagnosis of IgE-mediated FA based solely on clinical history often requires the documentation of allergen sensitization through in vitro allergy tests. sIgE testing is still considered as the most useful and practical tool in clinical practice. The BAT is a novel approach, not only capable of differentiating severe forms but, thanks to its high specificity, a useful tool in predicting FA as well, without the need to perform a food challenge. A proper selection of patients and therapeutic approaches would lead to the so-called precision medicine for a great impact on patients' quality of life and appropriate allocation of resources. On the other hand, IgG₄ testing is not considered as a useful test for the diagnostic workup of FA and should not be performed in allergic patients for diagnostic purposes.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare for the present work.

AUTHOR CONTRIBUTION

Davide Caimmi: Conceptualization (lead); Project administration (lead); Supervision (equal); Writing-review & editing (equal). **Stefania Arasi:** Methodology (lead); Writing-original draft (lead); Writing-review & editing (equal). **Simona Barni:** Methodology (lead); Writing-original draft (lead); Writing-review & editing (equal). **Carla Mastorilli:** Methodology (lead); Writing-original draft (lead); Writing-review & editing (equal). **Pasquale Comberiati:** Writing-review & editing (equal). **Fernanda Chiera:** Writing-review & editing (equal). **U. Pelosi:** Writing-original draft (equal); Writing-review & editing (equal). **Francesco Paravati:** Supervision (equal); Writing-original draft (supporting); Writing-review & editing (equal).

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