



Biomarkers in Food Allergy

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Abstract

Purpose of Review To familiarize the reader with the concept of precision medicine in food allergy by dealing with the current biomarkers for the diagnosis, prognosis, and management of the disease.

Recent Findings Many efforts have been devoted in order to characterize reliable biomarkers able to identify specific phenotypes and endotypes in food allergy. Specific IgE (sIgE), sIgE/total IgE ratios, and T cell assays are just a few candidates that have been investigated over time. With the advent of omics sciences, a new era is commencing. A better understanding of pathogenesis of food allergy and mechanisms of action of the different therapeutic options will allow the accurate selection of the appropriate patient.

Summary In the near future, advances in technologies and data interpretation will allow a better understanding of the pathogenesis of food allergy and the identification of proper biomarkers for a personalized treatment tailored on the specific patient's profile.

Keywords Biomarkers · Diagnosis · Food allergy · Omics · Precision medicine · Prediction

Introduction

To identify proper biomarkers is currently one of the main research targets throughout medicine, including food allergy [1]. Biomarkers are pivotal tools to enable a “precision medicine.” They can guide decisions with regard to the prediction, prevention, diagnosis, and treatment of illnesses in order to provide “the right treatment to the right patient, at the right dose at the right time.”

Food allergic reactions are responsible for a variety of clinical conditions mediated by an immunologic mechanism, involving specific IgE (IgE-mediated), cell-mediated mechanisms (non-IgE-mediated), or both IgE- and cell-mediated mechanisms (mixed IgE- and non-IgE-mediated) [2•]. This review will focus on IgE-mediated food allergy (FA). FA is a significant medical problem [2•]: the prevalence of this

potentially life-threatening condition is increasing over time similarly to other allergic diseases [3, 4]. Furthermore, it represents a kaleidoscopic disease. Although many patients may suffer from allergy to the same food, each of them is expected to display a specific allergic profile. Similar clinical phenotypes (in terms of severity and clinical history) may be associated to different sensitization patterns (type of allergenic molecules and respective specific IgE levels) and vice versa. Therefore, FA patients, who may incur potentially harmful dietetic limitations, will profit of “precision” diagnostics and treatment. They need to have predictive indexes for the severity of the disease and its natural history. This review aims to describe how biomarkers may help the clinician in the interpretation of the pathogenesis of FA, in its diagnosis, and in its management.

Biomarkers in Food Allergy Diagnosis and Prognosis

Currently, the FA diagnostic work-up encompasses a detailed clinical history and physical examination followed by in vitro or in vivo tests to detect IgE immunoreactivity against some specific allergens [2•]. In the cases of diagnosis unclear, an oral food challenge (OFC) is required [2]. It currently represents the gold standard test to provide a definitive diagnosis and, therefore, to prevent the patients from unneeded and potentially detrimental diets [21, 22]. Notwithstanding, OFC is

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Table 1 Biomarker candidates in food allergy

	Source				Clinical use			Notes
	Serum	Skin	Mucosa	Other	Diagnosis	Prognosis	Treatment	
Skin prick tests		X			X	X		Cutoff values may vary widely among studies [5–7]. These differences can result from the patient population (e.g., prevalence of FA, comorbidities) and/or heterogeneities among study protocols
sIgE (to allergen extracts)	X				X	X		Cutoff values may vary widely among studies [5–7]. These differences can result from the patient population (e.g., prevalence of FA, comorbidities) and/or heterogeneities among study protocols
sIgE (to allergen components)	X				X	X		The different stability of allergenic molecules may explain different patterns of allergenic reactivity according to the type and timing of food processing
sIgE (to allergen peptides)	X				X	X		Since food allergens contain linear and conformational IgE epitopes, the latter's allergenicity may be influenced by heat and other agents
sIgE/total IgE	X				X	X		Discordant data [8–11]
sIgG4/IgE	X				X	X		Discordant data [8–11]
BAT	X				X	X	X	Discordant data [11, 12]
MAT	X				X	X	X	Preliminary data show low sensitivity [13]
Mucosal biomarkers		X			X	X	X	Several cell types (e.g., CD103+ dendritic cells, CX3CR1+ macrophages, FoxP3+ regulatory T cells) interplay, involving different pathways [14, 15].
Omics sciences	X	X	X	X	X	X	X	They evaluate several analytes and cell components, such as proteins (proteomics), metabolites (metabolomics), transcripts (transcriptomics), genes (genomics), microbes (microbiomics), and environmental exposure (exposomics) [16]. In particular, epigenomics studies have revealed that the tolerance was associated with epigenetic regulation of the FOXP3 gene in allergen-specific regulatory T cells in patients undergoing OIT or spontaneously outgrowing their allergy [17, 18, 19]. The complexity of the data generated on large scale requires sophisticated mathematical integration
Integrated algorithms	X	X	X	X	X	X	X	Integrated approach combining different available diagnostic tools including lab values (CD63 ratio) and clinical variables (e.g., exercise-induced asthma and FEV1/FVC ratio) [20•]

BAT, basophil activation test; FEV1/FVC, forced expiratory volume in one second/forced vital capacity; MAT, mast cell activation test; sIgE, specific immunoglobulin E; sIgG, specific immunoglobulin G

logistically demanding and carries the risk of life-threatening anaphylactic reactions [23]. Hence, the need to perform OFC in clinical practice is widely debated and reliable prognostic markers for predicting the clinical outcome of this procedure are awaited [5]. A summary of the potential biomarkers is given in Table 1.

Skin Prick Tests and Serum Specific IgE to Allergen Extracts

Cutoff mainly for skin prick test (SPT) and specific immunoglobulin E (sIgE) has been widely investigated [6, 24, 25]. The proposed values of 0.35 kU/L and 3 mm, respectively for sIgE and SPT, have both high sensitivity but very low specificity, even lower than 20% in some studies [26–28]. However, they may be useful to exclude the diagnosis of FA as they have a high negative predictive value (NPV). Adopting 95% positive predictive value (PPV) cutoffs, the

specificity of SPT and sIgE assays increases, especially in the presence of a recent history of an immediate IgE-mediated reaction [7, 29]. However, diagnostic cutoff values may vary widely among studies [5–7]. These differences can result from the patient population (e.g., prevalence of FA, comorbidities) and/or heterogeneities among study protocols (e.g., inclusion criteria, reference standard against which the performance of SPT and/or sIgE was compared, criteria for referring for OFC and the OFC protocol) [5–7]. When comparing studies and when extrapolating cutoffs from published studies into daily clinical practice, these factors should be taken into account together with any further study limitation (e.g., small sample size, selected sample of participants, OFC not done in all participants). Validated diagnostic cutoffs can be used with discrete effectiveness when applied to the same population in which they were generated or a similar one. Outside of these situations, it has been suggested to use the likelihood ratio (LR), as it does not depend on prevalence of

the disease and offers a different diagnostic approach, practicable at the level of the individual patient [30].

Skin Prick Tests and Serum Specific IgE to Allergen Components

A big step forward in improving the accuracy of diagnosing IgE-mediated food allergy has been provided by the “molecular diagnostics” (MD). This allows to define the IgE sensitization pattern of a patient at molecular level through the use of purified, native, or recombinant allergen on singleplex or multiplex platforms [31, 32]. MD may identify the eliciting allergenic molecule and distinguish genuine sensitization from sensitization due to cross-reactivity (e.g., in pollen allergic patients tested for FA). Some IgE sensitization profiles at molecular level are linked to different patterns of clinical severity. For instance, sensitization to profilins and/or pathogenesis related protein-10 (PR-10) is usually related to mild allergic phenomena (oral allergic syndrome), while lipid transfer proteins (LTP), seed storage protein (SSP), and thaumatin are potentially responsible for more severe clinical patterns. This depends mainly on the stability of allergenic molecules, which may also explain different patterns of allergenic reactivity according to the type and timing of food processing. For instance, in most patients allergic to cow’s milk (CM) and hen’s egg (HE), caseins (Bos d 8) and ovomucoid (Gal d 1) sIgE levels have been identified as markers of reactivity (or tolerance) to heated CM and HE, respectively [33, 34]. The implications are highly relevant, even in terms of prognosis. It seems that the baked CM and HE allergy phenotype can strongly predict the likelihood of tolerance development [35]. Furthermore, sensitization to multiple egg allergens (i.e., Gal d 1, Gal d 2, Gal d 3, or Gal d 5) might be a prognostic marker that could be useful for identifying individuals at risk of developing persistent egg allergy [36]. It can be hypothesized that the specific patient’s molecular profile will be pivotal for developing a “precision” treatment.

Serum Specific IgE to Allergen Peptides

IgE specificity can be refined further by determining the allergen epitopes to which IgE binds. This has been evaluated using short linear allergen peptides of 15 up to 20 amino acids bound to a solid phase using immunofluorescence [37]. Some studies are testing the utility of allergen peptides in diagnosing and in predicting the resolution of FA [38, 39].

Since food allergens contain linear and conformational IgE epitopes, the latter’s allergenicity may be influenced by heat and other agents. Peptide microarrays might be useful in identifying different phenotypes of FA, for instance patients reacting to baked milk compared to patients reacting to raw milk but tolerating baked milk [40]. However, further studies are needed.

Ratios (Allergen-Specific/Total IgE and Allergen-Specific IgG4/IgE) and Functional Assays

To try to improve the diagnostic and prognostic performance of food sIgE, the added value of total IgE and food-specific IgG4 has been tested in food-specific/total IgE ratios or food-specific IgG4/IgE ratios [8–10]. Researchers have taken this path both for allergies triggered by protein-antigens [8–10] and for allergies triggered by alpha-Gal terminated saccharides [11]. Notwithstanding, the added value of these parameters in the prediction of both OFC outcome and history of the disease is still widely debated as data are discordant [41, 42].

The basophil activation test (BAT) is a functional assay that uses live basophils in whole blood to detect the ability of IgE to mediate activation of basophils after stimulation with allergen [12]. More recently, it has been investigated whether the ability to elicit peanut-induced cell activation could be transferred by passive sensitization of LAD2 mast cells with patients’ plasma (mast cell activation test, MAT) [13]. Both test showed low sensitivity (73%) in predicting the OFC outcome [13]. However, BAT allowed a good differentiation between patients with alpha-gal syndrome and asymptomatic alpha-gal sensitization (sensitivity 90%, specificity 100%) [11].

Omics Sciences

Recent technological advances have made it possible to move towards sophisticated high-throughput approaches (indicated by the suffix -omics), overcoming the limitations of the traditional biological techniques [16, 43]. Specifically, the different omics technologies nowadays find application for the simultaneous characterization and quantification of a wide spectrum of analytes and cellular components from a single biological sample, including proteins (proteomics), metabolites (metabolomics), transcripts (transcriptomics), genes (genomics), microbes (microbiomics), and environmental exposure (exposomics) [1, 16]. Omics technologies are now used commonly in diverse areas of research because of their increasing availability and decreased costs. However, the complexity of the data generated on large scale and the necessity of a mathematical integration represent the new fascinating challenge for the researchers.

Integrated Algorithms

Likely, an integrated approach combining different available diagnostic tools might achieve a more precise predictive performance. An algorithm has been recently developed in order to identify subjects at higher risk for severe reactions to a standardized peanut OFC. Authors included lab values (CD63 ratio) and clinical variables (e.g., exercise-induced asthma and FEV1/FVC ratio) [20•]. Further larger and multi-center studies are needed before exporting it outside of the

specific research settings. With the advent of omics sciences, we are already into the era of “big data”: researchers are called to generate algorithms integrating data from different sources and technologies to perform a “precision” diagnosis.

Mucosal Biomarkers

Recent research has advanced understanding of the immunological mechanisms mucosal occurring during the effector phase of allergic reactions to ingested food at local and systemic level. Several cell types are involved, including CD103+ dendritic cells (DC), CX3CR1+ macrophages, and FoxP3+ regulatory T cells (Treg) [14]. Interactions among these cells may contribute to an individual’s susceptibility to IgE-mediated food allergy [15] and a better understanding could allow a more precise therapeutic approach (see below).

Biomarkers in Food Allergy Treatment

To characterize biomarkers for identifying specific phenotypes and endotypes is pivotal in order both to improve the diagnostic accuracy and, then, to select the proper treatment for each patient. Currently, FA management consists of educating the patient to avoid ingesting the culprit allergen and to initiate therapy in case of an unintended ingestion [2••]. However, an elimination diet may be difficult and frustrating, above all for those foods that are pivotal in the common diet (e.g., CM and HE). Nevertheless, despite efforts to comply with this diet, accidental exposures leading to adverse reactions occur frequently [44].

Novel strategies are under investigation. Oral immunotherapy (OIT) may modify the natural history of the disease as a potential curative treatment [45]. It can increase the threshold of reaction while on OIT treatment in children with persistent HE or CM allergy [46]. However, there are still some gaps to be filled [47••, 48]. There is lack of evidence concerning the continued effectiveness after FA-AIT discontinuation. Specific and sensitive biomarkers to predict the response to the treatment are missing. A better understanding of the mechanisms underlying OIT might overcome these limitations. At this regard, there is evidence that T cell responses are crucial to develop oral tolerance in non-allergic individuals and the allergic immune response in allergic individuals. It has been shown that patients with peanut allergy (PA) have greater proliferation of their allergen-specific T cells when their PBMCs have been stimulated with whole peanut or individual major peanut allergens [49, 50]. Moreover, PA patients have shown a typical Th2-skewed response to peanut allergen whereas non-allergic controls showed a Th1-type response [49, 50]. Interestingly, peanut allergic and peanut-sensitized-tolerant individuals showed higher T cell proliferation compared with non-sensitized subjects; however, only allergic patients showed a Th2-skewed

response to peanut allergens [50]. These findings suggest that the absence of clinical reactivity in IgE-sensitized individuals is an active ongoing process, whereas in non-sensitized individuals, it is a passive process, probably due to anergy or clonal deletion [50]. Food allergic patients may also have impaired regulatory T cell function in response to specific food allergens. It has been assessed that egg and/or peanut allergic infants had a reduction in the number of Tregs and a lower ratio of activated regulatory/effector T cells in vitro after in vivo allergen exposure during the OFC [51]. The novel mass cytometry techniques [52] are currently used to characterize allergen-specific T cells over OIT [53]. Epigenomics studies have revealed that the tolerance was associated with epigenetic regulation of the FOXP3 gene in allergen-specific regulatory T cells in patients undergoing OIT or spontaneously outgrowing their allergy [17•, 18, 19]. In patients with FA, successful OIT in patients with peanut allergy has been associated with conversion of allergen-specific T cells to anergic T cells [54]. These findings, along with technological developments that allow real-time monitoring, could have therapeutic implications and enable personalized medicine. In this perspective, reliable biomarkers for a standardized classification of the severity of the disease are also needed [55–57]. Most patients undergoing OIT experience side effects, usually mild; however, they could be severe in children with severe FA. Engineered food allergens with altered IgE epitopes hold the promise to improve the safety—and, therefore, also the efficacy—of AIT [58]. Similarly, in murine models, the co-administration of ovalbumin with cholera toxin as adjuvant able to reduce intestinal epithelial cell (IEC)-derived eotaxin could be a potential strategy to limit the severity of allergic responses to food antigens [59]. Notwithstanding, patients with severe food allergy are also likely to have allergies to multiple food, whereas OIT is strictly food specific and would require consecutive time-consuming protocols [60]. For these patients, a better therapeutic option might be biologicals, such as omalizumab (anti-IgE monoclonal antibody), in association with OIT [61, 62] or alone [63].

Further integrative options are currently under investigation for a FA management tailored on the single patient. Data support the hypothesis that bioactive peptides in cow’s milk hydrolysates would carry out not only a passive role in CM allergy but even an “active” one with possible immunomodulation properties [64]. The intestinal microbiome is considered as a metabolically active “organ” affecting the host’s metabolic capabilities, immunity, and pathogenesis [65, 66]. The study of the entire proteome of microbiota (metaproteome) might provide information on the real activities in microbial communities [67]. In the near future, it is expected that the microbiome characteristics, as potential biomarkers of specific endotypes, might open the way to a personalized nutritional and/or medical intervention.

Furthermore, the absolute quantification and characterization of allergens—including structural modification, degradation in

the gastrointestinal environment, or identification of suitable marker peptides for the development of novel analytical approaches—will help not only the allergic consumer and his/her caregiver to assess the risk of allergen levels of a food product but also preliminary for the production of safer foods from manufacturers. A correct food labeling will increase the chance to be successful in providing a safe and high-quality food [68, 69].

Conclusions

FA is a complex and heterogeneous disease. Specific and sensitive biomarkers are essential tools for selecting the proper treatment for the proper patient and enabling a “personalized medicine.” Many steps forward have been recently done in this direction. With these fast-growing efforts to generate and integrate data from different technologies, it is not difficult to imagine that in the near future, we will be able to better understand the cause and mechanisms of FA and devise better ways to treat and perhaps even cure this disease, providing a therapeutic approach targeted to the patient.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Abbreviations BAT, basophil activation test; CM, cow’s milk; FA, IgE-mediated food allergy; FEV1/FVC, forced expiratory volume in one second/forced vital capacity; HE, hen’s egg; IEC, intestinal epithelial cell; LR, likelihood ratio; LTP, lipid transfer proteins; MAT, mast cell activation test; MD, molecular diagnostics; MS, mass spectrometry; NPV, negative predictive value; OFC, oral food challenge; OIT, oral immunotherapy; PA, peanut allergy; PR-10, pathogenesis-related protein-10; PPV, positive predictive value; sIgE, specific immunoglobulin E; SPT, skin prick test; Treg, regulatory T cell

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- Of importance
- Of major importance

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