Precision Allergy Molecular Diagnosis in Predicting Atopy Development

Subjects: Allergy

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The allergic march is a progression of naturally occurring symptoms whose nature changes with age. The classic allergic march typically begins in infancy and manifests in the form of atopic dermatitis and food allergy. As immune tolerance develops over time, these conditions may resolve by the age of 3–5 years; however, they may evolve into allergic rhinitis and bronchial asthma. Traditional diagnostic assessments, such as skin prick testing or serum allergen-specific immunoglobulin E (sIgE) level testing, are conducted to introduce effective treatment. The precision allergy molecular diagnosis (PAMD@) assesses sIgE against allergenic molecules. This new technology helps more accurately evaluate the patient's allergy profile, which helps create more precise dietary specifications and personalize allergen-specific immunotherapy.

precision allergy molecular diagnostic applications | PAMD@ | allergic march | %MCEPASTEBIN% | allergy molecular diagnosis | component resolved diagnostics (CRD)

1. Introduction

Allergy that occurs in the first years of a child's life is typically food allergy (FA) [1]. Due to its increasing incidence, FA is an important problem in clinical pediatrics. FA may manifest with mild symptoms, such as abdominal discomfort, nausea, vomiting, or diarrhea, but also with severe, life-threatening symptoms, which may be due to immunoglobulin E (IgE)-mediated hypersensitivity (e.g., anaphylactic shock) or due to severe dehydration and electrolyte imbalance resulting from intense vomiting or diarrhea caused by non-IgE-mediated reactions [2][3]. Hypersensitivity to foods is often also the first step in the so-called allergic march—a progression from FA to inhalant allergy, which leads to asthma [4]. Usually, one of the first FA manifestations is atopic dermatitis (AD), which is a common condition in early childhood, with an estimated prevalence of 15–25% in children [5]. Data from the literature show that approximately 45% of infants develop AD symptoms before the age of 6 months, with the proportion rising to 50% by the age of 1 year. Comorbid IgE-mediated FA and AD in infancy and early childhood are the earliest manifestations of the atopic march [6][7]. Symptomatic FA, especially severe or multiple ones, was shown to be closely associated with bronchial asthma in children aged ≥6 years [8]. Children with FA developed bronchial asthma earlier than children without this allergy [9]. Another study revealed that milk sensitivity in infancy predisposes the child to severe respiratory tract infections and airway hypersensitivity to histamine [10].

2. PAMD@ Assays

The means of determining the cause of allergy in routine laboratory diagnostics involve measuring the levels of sIgE against the most common allergens (including allergens of foods, such as cow's milk, eggs, wheat, soy, nuts, and fish, and inhalant allergens, such as birch, timothy grass, house dust mites, molds, and animal allergens) [11]. Developed several years ago, PAMD@ is a state-of-the-art form of allergy diagnostics, which helps establish the allergy type (primary/cross-reaction), course (depending on the type of protein allergens), and prognosis (transient/persistent allergy) [11][12]. The use of PAMD@ makes it possible to identify individual allergen molecules and assess them comprehensively via multiplex testing.

Singleplex PAMD@ is used for assessing the serum levels of sIgE against individual allergenic molecules. Depending on the technical characteristics (solid-phase and liquid-phase assays, various solid-phase substrates, native and recombinant components, different types of detection antibodies, and different types of enzyme interactions), the tests have varied sensitivity and specificity. Singleplex tests yield quantitative results but require the use of relatively large serum sample volumes (40–50 µL of serum per allergen, plus the so-called dead space volume of approximately 100 µL), and their cost per single assay is relatively high [13]. Conversely, multiplex PAMD@ involves the simultaneous determination of sIgE for multiple allergen components in a single assay. The first multiplex assay was ImmunoCAP ISAC, capable of analyzing sIgE against a total of 112 allergen components from 50 allergen sources. This was followed by the emergence of third-generation nanotechnology applications. The first of such assays was FABER (which is now no longer produced), capable of simultaneously analyzing 122 molecules and 122 allergen extracts, and another was ALEX (after changes in 2019—ALEX2), capable of analyzing 178 allergen molecules and 117 allergen extracts. One unquestionable advantage of ALEX2 tests over other assays is the presence of a cross-reactive carbohydrate determinant (CCD) inhibitor, which greatly reduces false-positive results. Moreover, unlike the ImmunoCAP ISAC assay, which is semi-quantitative, ALEX2 is a quantitative assay [11].

3. Allergy Prognosis Based on PAMD@

3.1. PAMD@, Allergy Symptoms, and Provocation Testing

PAMD@ seems to be useful in predicting the type and severity of allergic symptoms. In the case of cow's milk allergy, determining the sIgE to individual allergen components helps identify patients allergic to casein (Bos d 8), who are at a high risk for anaphylactic reactions, and those allergic to alpha-lactalbumin (Bos d 4) or beta-lactoglobulin (Bos d 5), whose risk of severe anaphylaxis is lower and who can be expected to develop milder symptoms, mainly in the form of skin lesions or gastroenteritis [11]. Another example is egg allergy, where the detection of sIgE against ovomucoid (Gal d 1), which is an egg protein, is associated with high risk of anaphylaxis [11]. PAMD@ can also be used in predicting the results of allergen provocation tests. Depending on the type of test used, the levels of casein-specific IgE that have been reported to be predictive of a positive oral cow's milk provocation test range from 0.95 kU/L to 10.0 kU/L [14][15][16][17].

Moreover, PAMD@ helps predict if the allergy is temporary or persistent. In a prospective study, Dang et al. determined the levels of sIgE to egg molecules (Gal d 1, 2, 3, and 5) and to an egg protein extract in three

subgroups of 12-month-old infants [18]. These subgroups were infants with egg white allergy confirmed via allergen provocation testing, infants with egg sensitivity, and those with egg tolerance. The study was followed up at the ages of 2 and 4 years and showed that Gal d 1 sensitization increased the risk of long-term egg allergy five-fold, and the presence of sIgE to all egg allergens (Gal d 1, 2, 3, and 5) increased the risk of persistent allergy to raw eggs four-fold.

3.2. Assessment of Molecular Spreading

PAMD@ also helps us observe the phenomenon of molecular spreading, which involves progressive sensitization to other allergenic molecules from a given source in patients initially sensitized only to a single type of allergenic molecules. This phenomenon was described by Matricardi et al. who evaluated the course of sensitization to timothy grass (*Phleum pratense*) in a boy from the age of 3 to 10 years. Initially, at the age of 3 years, the boy was diagnosed only with sensitivity to Phl p 1 [19]. Subsequently, at the age of 6, he was also found to have Phl p 2 sensitivity. By the age of 10 years, the boy had become sensitized to Phl p 4, Phl p 5, Phl p 6, and Phl p 11 molecules. The authors of that study hypothesized that the introduction of allergen-specific immunotherapy would stop or inhibit this molecular spreading and the associated progression of allergy symptoms [19]. Posa et al. followed up with pediatric patients to evaluate the extent of their allergy by analyzing the sIgE against molecules of *Dermatophagoides pteronyssinus* over a period of 20 years [20]. The most common molecules (>40%) detected early in their lives were Der p 2, Der p 1, and Der p 23 (molecules of group A), followed by (15–30%) Der p 5, Der p 7, Der p 4, and Der p 21 (molecules of group B). The least common (<10%) sensitivities were to Der p 11, Der p 18, Der p 16, Der p 14, and Der p 15 (molecules of group C). Sensitization usually started with group A proteins. Over time, blood tests revealed the presence of sIgE to group B allergens and, eventually, to group C molecules. Early-onset sensitization, extensive exposure to house dust mites, and parental allergic rhinitis were associated with the development of overtly symptomatic allergy during the subsequent years. The patients sensitized to all house dust mite allergen groups listed above (groups A + B + C) were at a significantly higher risk of allergic rhinitis and bronchial asthma. The presence of serum sIgE against Der p 1 or Der p 23 at the age of 5 years or younger was a positive prognostic factor for the development of asthma by school age [20].

4. PAMD@ and Allergen-Specific Immunotherapy

The data verified based on the PAMD@ results can be invaluable in preparing a patient for immunotherapy [21]. Such data help personalize the immunotherapy vaccine, which improves the effectiveness of the entire course of immunotherapy and increases the chances for successful management of atopy [22]. Diagnosing allergy based on allergenic molecules also helps assess if the patient's hypersensitivity is associated with the so-called true allergy or cross-reactivity, which may be very important in making decisions on causative treatment.

Until recently, the selection of vaccines for allergen-specific immunotherapy was very challenging in patients with both symptomatic allergic rhinitis in the season from March to June and the presence of sIgE against birch and timothy allergen extracts [23].

The content of whole-allergen extracts is highly variable. They may not even contain clinically significant allergen components, which may pose diagnostic difficulties and lead to suboptimal immunotherapy vaccine selection. Frick et al. evaluated Api m 1 and Api m 10 levels in commercially available whole-allergen extracts used for bee venom immunotherapy [24]. The absence of Api m 10 in the extract (found in 3 out of 5 analyzed samples) was associated with a ten-fold higher risk of immunotherapy failure. Determining the Api m 10 levels and, possibly, the use of an allergen extract containing Api m 10 are indicated in clinical practice in patients who have failed to respond to immunotherapy, which is supposed to protect the patient against an anaphylactic reaction to a bee sting [24].

It is also possible for a patient to present with obvious allergy symptoms but have undetectable serum sIgE levels or negative skin prick test results. Nonetheless, once individual allergen molecules are analyzed, the final diagnosis may be sensitivity to a molecule that is completely absent from standard whole-allergen extracts or whose levels in those extracts are very low. This may be due to the methods used to manufacture the test extract or to the physicochemical properties of the given allergen [25], which can be exemplified by Api m 10 components (which play an important role in bee venom immunotherapy) or Der p 10 and Der p 23 components (which play a role in house dust mite allergy) [26].

5. The Lower Limit of Normal sIgE Levels in PAMD@

The discovery of IgE by Ishizaka and by Johansson and Bennich reported in 1960 was an important milestone in allergy diagnosis [27]. Another important milestone was a method of detecting the serum levels of IgE against individual allergens. Sensitivity and specificity analysis of the diagnostic tests available in the 1970s helped establish the lower limit of normal sIgE levels to be 0.35 kU/L [28]. The issue of lowering this lower limit below 0.35 kU/L has been recently suggested and discussed [29]. Nilsson et al. demonstrated that food allergen-specific IgE levels of 0.1–0.34 kU/L (in the case of allergens such as eggs, milk, or nuts) detected in infancy increase the risk of developing inhalant allergies at the age of 5 years, and—in the case of low levels of egg-specific IgE—also the risk of developing AD in early childhood [30]. Therefore, sIgE levels in the first year of life, despite being below 0.35 kU/L, may be an additional predictive factor of allergy development.

Clinically evident allergic disease appears to be present when the IgE antibody level is greater than 0.35 kU/L, but not always, and it depends highly on the specific-to-total IgE ratio, the total IgE of the patient, the extent of allergen exposure, and the “sensitivity” of the patient’s mast cells. Nevertheless, the interpretation of sIgE results in the range of 0.1 to 0.35 kU/L should be made with caution.

6. PAMD@ Not for Everyone

Currently, PAMD@ is not meant to be part of routine allergy diagnostics. Disease-management protocols, including those on patient eligibility to undergo immunotherapy, still require positive allergen extract-based sIgE or positive skin prick test results [31]. Nonetheless, this method serves the additional function of helping to make the results more precise. PAMD@ is not intended for monosensitized patients with a predictable, seasonal pattern of allergy

symptoms. These patients require only routine diagnostic tests involving sIgE levels or skin prick testing in order to receive immunotherapy.

With the development and greater availability of PAMD@, practical questions of clinicians are: “how to” and “when” to use molecular allergen diagnosis and whether such a diagnostic strategy is appropriate in terms of costs and predictive values. There is no single optimal answer to these questions. Each case should be considered individually based on the clinical condition of the patient. Nevertheless, scholars can identify certain groups of patients who benefit most from the use of PAMD@ in the process of diagnosing their clinical symptoms. These are subjects classified for immunotherapy (patients allergic to single or several inhalant allergens, patients with multiple allergies to pollen or with allergy to *Hymenoptera insects*), patients with anaphylaxis (after food, with the participation of cofactors, with delayed anaphylaxis after red meat, idiopathic anaphylaxis), patients with latex allergy, with polysensitization (especially those with a co-existence of sensitization to inhalant and food allergens), and patients with food allergy (to assess the risk of the severity of allergic reactions and to identify unexpected sources of sensitization). At this point, the importance of clinicians should also be emphasized. They are responsible for deciding when and which diagnostic strategy should be used, taking into account the patient’s symptoms and the local law.

7. Limitations of PAMD@

One of the limitations is the number of molecules marked with PAMD@. Currently, over a thousand molecules are described, but scholars can routinely label fewer than 200 of them. Due to cost constraints and the clinical value of the results, only very few component sIgE antibodies are routinely run on singleplex assays today.

Another limitation is that some important molecules cannot be determined otherwise than by means of multiplexes [11][32]. One example of such proteins is oleosins, which have been shown to be important in patients who have a history of anaphylaxis after consuming peanuts, sunflower seeds, or soy and who have skin or blood tests negative for allergen extracts. The determination of proteins from this group allows for the assessment of the risk of severe anaphylaxis and the clarification of ambiguous cases of allergy [32].

As in the case of allergen extract-based diagnostics, the results of PAMD@ may not always be consistent with clinical manifestations. One of the possible explanations for this phenomenon involves the CCDs that are present in some proteins [11][33]. Anti-CCD antibodies may produce positive results in in vitro allergy tests, which may hinder the clinical interpretation of laboratory test results. This problem may affect up to 30% of patients.

8. Summary

Using PAMD@ in allergy diagnostics and reducing the lower limit of normal sIgE levels are intended to diagnose allergies as early as possible and help assess the risk of molecular spreading and anaphylaxis. PAMD@ is also a state-of-the-art tool that helps to make decisions on the introduction of causative treatment—allergen-specific immunotherapy and personalized selection of immunotherapy vaccines. The actions taken based on the

information obtained via PAMD@ may help stem allergy development. Considering the substantial usefulness of PAMD@ in the clinical management of patients with inconclusive results of routine allergy tests, having this technology at our disposal makes allergic march control seem more achievable.

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