



# Capabilities of Molecular-Based Allergen Diagnostics in Atopic Diseases

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## Abstract

One hundred thirty-one assays were performed to determine the level of specific IgE to major molecular components of the birch pollen—rBet v 1, timothy grass pollen—rPhl p1, rPhl p 5b, and house dust mites—nDer p1, rDer p2 as well as to the minor components of birch pollen—rBet v 2, rBet v 4, timothy grass pollen—rPhl p7, rPhl p 12, and house dust mites rDer p10 in patients with asthma ( $N = 66$ ) and allergic rhinitis ( $N = 65$ ). It is well established that sensitization to major and minor allergens facilitates the selection of an allergen for the allergen-specific immunotherapy (ASIT). According to the concept of molecular-based allergen diagnostics, the ASIT efficacy is expected to be high when IgE to major components are present and antibodies to minor components are absent. When elevated IgE levels to major and minor ASIT proteins are present, a moderate ASIT efficacy is expected.

**Keywords** Molecular-based allergen diagnostics · Major allergen · Minor allergen · Allergen-specific immunotherapy

## 1 Introduction

Molecular identification of allergen components involved in the atopic and autoimmune diseases became possible with the practical application of biotechnologies. Panels of recombinant and natural molecules for various allergens have been developed (tree, grain and weed pollens, house dust mites, food-borne allergens, and pet dander) and used for diagnostic. Natural extracts used in a routine practice contain large number of epitopes. Therefore, utilizing molecular components of each allergen increases the sensitivity and specificity of diagnostic tests. Molecular (component) diagnostics utilized in the ImmunoCAP technology (Phadia, Sweden) has been proven valuable to identify the allergen. Molecular-

based allergy (MA) diagnostics is an approach used to map the allergen sensitization of a patient at a molecular level, using purified natural or recombinant allergenic molecules (allergen components) instead of allergen extracts (A WAO-ARIA-GA<sup>2</sup>LEN consensus document on molecular-based allergy diagnostics) [1, 2]. The most important aspects of MA diagnostics are ability to identify a causative molecule, discriminate specific and cross-reactive molecules and capability to determine the structure of the allergen [3].

One of the most important aspects of MA is the evaluation of the effectiveness of allergen-specific immunotherapy (ASIT) in patients with seasonal and allergic rhinoconjunctivitis, atopic bronchial asthma, and anaphylactic reactions [4–6]. MA of the sensitization profiles improves the selection of patients for the ASIT and minimizes the use of ASIT in patients with polysensitization (sensitization to three or more groups of allergens) [6–8]. Recent study has shown that the use of MA improved the ASIT results when compared to prick tests [5]. Although the MA is currently used in clinical allergy diagnosis, further studies are needed to select the categories of patients for ASIT.

The ImmunoCAP (Phadia) technology, which is used for MA, is designed to detect ultra-low concentrations of immunoglobulins and inflammation markers in extremely small sample size. Technically, this is achieved by using a solid

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**Table 1** Major and minor allergen components used to predict ASIT efficacy

| Allergens       | Major allergen components |                                         | Minor allergen components |                                  |
|-----------------|---------------------------|-----------------------------------------|---------------------------|----------------------------------|
|                 | Allergenic molecule       | Belonging to superfamily                | Allergen component        | Belonging to protein superfamily |
| Trees           | rBet v 1                  | PR-10 pathogenic proteins               | rBetv2<br>rBetv4          | Profilin<br>Ca-binding protein   |
| Timothy         | rPhl p 1, rPhl p 5b       | PR-10 pathogenic proteins               | rPhl p 7<br>rPhl p 12     | Ca-binding protein<br>Profilin   |
| House dust mite | nDer p 1<br>rDer p 2      | Cysteine protease<br>NPC family protein | Der p 10                  | Tropomyosin                      |

phase of a porous material (a unique development of the company Phadia) containing the immobilized antigens. Due to the porous three-dimensional structure of the solid phase, the sorption capacity of the system increases 150 times as compared to the conventional immunosorbent assay. The lower limit of asIgE detection of the Phadia systems is 0.1 kU/l. The unique technology for ultra-precise allergodiagnosics, Phadia's reagents and automatic analyzers, is currently recognized as the "Gold Standard" and is a reference system that has no analogues in the world. The MA method with the help of allergen components is by far the most progressive tool among the tests used for allergy diagnostics in vitro. The sensitivity (the degree of detection of positive samples from positive) of the method is 93%, and the specificity (the degree of detection of negative samples from negative ones) is 89%. At present, the patient is considered as sensitized to an allergen if the concentration of antibodies exceeds 0.35 kU/l. The lower limit of detection of the device is much smaller values, for example, cat dander (e1)—0.06 kU/l, cow milk (f2)—0.05 kU/l, egg white (f1)—0.08 kU/l, and warty birch (t3)—less than 0.01 kU/l. All tests are performed in a fully controlled environment at a stable temperature, which allows for high reproducibility of the results [1, 2].

Recognition of the triggering allergens is essential for specific immunotherapy, resolving cross-reactive sensitization in polysensitized patients and identifying the primary sensitizer.

The study was aimed to present our experience in identifying allergen using molecular-based allergen diagnostics in atopic diseases and to evaluate the efficacy of allergen-specific immunotherapy (ASIT).

## 2 Materials and Methods

Children and adults with atopic bronchial asthma (ABA) ( $N=66$ ) and allergic rhinitis (AR) ( $N=65$ ), with polyvalent sensitization (sensitization to three or more allergens) and/or with discrepancy in the results of allergic anamnesis and skin testing, were enrolled in this study. ABA has comorbidity with allergic persistent rhinitis (47%), allergic conjunctivitis (39.4%), and oral allergy (36.7%). AR was accompanied by allergic conjunctivitis (80%) and symptoms of oral allergy (60%). Patients included in the study were sensitized to house dust mites (46.6%), pollen trees (74.8%), and pollen grass (57.3%).

MA was conducted at the third stage of allergological examination of patients after careful collection of allergological anamnesis (seasonality or year-round clinical symptoms, periods of the most severe symptoms, the presence and severity of clinical manifestations, the influence of meteorological conditions and the patient's living conditions, the presence of oral allergic syndrome), the setting of tests with domestic extracts domestic, pollen, and allergenic animals, and the definition of asIgE for inhalation allergens (routine immunoassay

**Table 2** Prognosis of ASIT efficacy with birch allergen

| ASIT efficacy | Bet v 1 «+»          | Bet v 1 «+»          | Bet v 1 «-»              |
|---------------|----------------------|----------------------|--------------------------|
|               | Bet v 2, Bet v 4 «-» | Bet v 2, Bet v 4 «+» | Bet v 2, Bet v 4 «+»/«-» |
|               | High                 | Moderate             | Low                      |

**Table 3** Prognosis of ASIT efficacy with timothy grass pollen

|               |                                                          |                                                             |                                                             |
|---------------|----------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|
| ASIT efficacy | Phl p 1, Phl p 5b; «+»<br>Phl p 7, Phl p 12; «-»<br>High | Phl p 1, Phl p5b; «+»<br>Phl p 7, Phl p 12; «+»<br>Moderate | Phl p 1, Phl p 5b; «-»<br>Phl p 7, Phl p 12; «+»/«-»<br>Low |
|---------------|----------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|

analysis with using the test systems of the firm “Alcor-Bio,” Russia). Detection of asIgE to allergens (Phadia/Thermo Scientific) was carried out using the Phadia-100 analyzer (Sweden, Phadia/Thermo Scientific), allergic components of birch pollen—rBet v 1, rBet v 2, rBet v 4 ( $N=64$ ), timothy pollen—rPhl p1, rPhl p 5b, rPhl p7, rPhl p 12 ( $N=43$ ), home dust mite-cysteine protease nDer p1, protein of the family NPC2-rDer p2, rDer p10 ( $N=24$ ).

The result was interpreted as follows:

- 0- An undetectable level  $< 0.35$  kUA/l;
- 1- A very low antibody level— $0.35-0.7$  kUA/l;
- 2- A low antibody level— $0.7-3.5$  kUA/l;
- 3- A positive (moderate) level— $3.5-17.5$  kUA/l;
- 4- A high level— $17.5-50$  kUA/l;
- 5- A very high level— $50-100$  kUA/l;
- 6- A level above the limit of detection  $> 100$  kUA/l.

Patients were subjected to ASIT sublingual method. Patients received the pre-seasonal course with pollen allergens of trees or meadow grasses in case of seasonal allergic manifestations or year-round course of house dust mite in case of sensitization to house dust mite.

Statistical analysis was conducted using Prisma v.5 software with the calculation of a relative incidence value for a characteristic in percent (intensive coefficients, IC) as well as 95% confidential interval of its true value (95% CI).

### 3 Results and Discussion

The incidence rate of allergen components in MA diagnostics was commonly described as “major” and “minor” [1, 2, 5, 6]. Major allergen components (M) are allergenic molecules which are characterized by the presence of antibodies in more than 50% of patients in the population with the same allergy (M; Table 1). Allergens with the incidence rate of less than 10% are considered as minor (m; Table 1). It should be noted

the classification of allergens into M and m depends entirely upon the sensitization profile of the population studied and allergen sources prevailed in a given geographical region [6].

One of the most important aspects of MA diagnostics is the ability to predict the efficacy of allergen-specific immunotherapy (ASIT). The effectiveness of ASIT is determined by decreased level of specific IgE and increased content of allergen-specific blocking antibodies of IgG class. Antigen-specific IgG-IgE antagonists block the cascade of allergic inflammation that occurs upon the contact of IgE with an allergen. The change in the balance of IgG4 and IgE decreases the sensitivity to allergens until full tolerance is achieved.

ASIT restores the immune system balance and reduces the sensitivity to allergens until complete tolerance is developed. It reduces the allergy symptoms and the use of drugs as well as prevents polysensitization and progression of the allergic rhinitis into the bronchial asthma. Due to its long-term effect, ASIT is considered a therapy which can cease the further development of allergy and disease progression. ASIT is the most successful when disease is timely diagnosed; the full range of triggering allergens is identified and therapeutics are administered regularly. ASIT is an expensive treatment where the identification of a causative allergen is essential for its success [6, 7].

It is difficult to select an allergen for ASIT in patients with polysensitization detected by traditional diagnostic tests using allergen extracts and when clinical history and skin testing data are not sufficient to identify a causative allergen. According to the concept of MA diagnostics, the efficacy of ASIT is expected to be high with an elevated level of IgE to M and lack of antibodies to m components. When IgE to M and m proteins are present at the same time, ASIT may be ineffective. When IgE to m proteins are present, while antibodies to M proteins of the allergen are absent, ASIT is not recommended. At present, various recombinant (r) and native (n) allergen components are being used in allergology, worldwide [7, 8].

Allergen components such as tree (birch) pollen—rBet v 1, rBe tv 2, rBet v 4, grass pollen (timothy)—rPhl p1, rPhl p 5b,

**Table 4** Prognosis of ASIT efficacy with house dust mite

|               |                                                 |                                                     |                                                |
|---------------|-------------------------------------------------|-----------------------------------------------------|------------------------------------------------|
| ASIT efficacy | nDer p1, rDer p 2; «+»<br>rDer p10; «-»<br>High | nDer p1, rDer p 2; «+»<br>rDer p10; «+»<br>Moderate | nDer p1, rDer p 2; «-»<br>rDer p10; «+»<br>Low |
|---------------|-------------------------------------------------|-----------------------------------------------------|------------------------------------------------|

rPhl p7, rPhl p 12, and house dust mites—nDer p1, rDer p2, rDer p 10 are most often used to predict the efficacy of ASIT with different extracts (Tables 2, 3, 4).

When selecting an allergen for ASIT, it is important to consider clinical symptoms, when most severe symptoms are observed, symptom duration, and their effect on a patient's quality of life, as well as which allergen can be most difficult to avoid. The detection of sensitization to major and minor allergens helps the selection of an allergen for ASTI in patients with polysensitization, complex clinical history, and results of skin testing with allergen extracts [2, 7, 8].

We have performed panel molecular assays of antibodies to components of birch, timothy grass pollen, and house dust mites in order to predict the ASIT efficacy; the results are provided in Table 5.

Panel molecular sensitization assays to birch pollen components were performed in 64 patients. Majority (90.6%) of patients had elevated level of specific antibodies to the major birch pollen molecular component—rBet v 1 ( $<0.35$  kUA/l in 9.37% of cases). There was no class 1 level antibody detected, while level of class 2 antibodies was found 15.62%. Class 3 antibodies were found in 3.13% patients, class 4 antibodies were detected in 34.38% patients, class 5 antibodies were demonstrated in 18.75%, and class 6 antibodies were found in 18.75% patients. We have found that 18.75% of patients were sensitized to minor birch pollen molecular components. Among these patients, 9.37, 3.13, and 3.12 had class 3, 4, and 5 antibody levels, respectively. These data indicate that high level of antibodies to the major birch component rBet v 1 ( $>100$  kUA/l) was prevalent, with moderate sensitization to the minor component rBet v 2.

Panel molecular sensitization assays to timothy grass pollen were performed in 43 patients. The majority (90.7%) of patients had elevated level of specific antibodies to the major timothy grass pollen molecular component (rPhl p1, rPhl p 5b). When antibodies to different antigen classes were analyzed, 9.3% of cases had  $<0.35$  kUA/l; the class 1 level was 9.3%, class 2—18.6%, class 3—34.88%, class 4—16.28%, and class 5—11.63%. Moderate and high antibody levels were prevalent among reactivity to the major timothy grass pollen components. Patients (18.6%) were also sensitized to minor timothy grass pollen components (rPhl p7 rPhl p 12), and no class 1 and 2 levels were detected; class 3 and 4 levels were observed in 9.3% of patients. The moderate and high level sensitization to minor timothy grass pollen components was prevalent.

Panel molecular sensitization assays to the house dust mite molecule components were carried out in 24 patients. Increased level of specific antibodies to cysteine proteinase (nDer p1) was detected in 65.66% of patients. No one had the result of  $<0.35$  kUA/l; the class 1 level was 8.33%, class 2—8.33%, class 3—8.33%, class 4—24.0%, class 5—8.33%, and class 6—8.33% of cases. Majority of patients (90.66%)

**Table 5** Panel molecular antibody assays to components of birch, timothy grass pollen, and house dust mites

| Level                            | Birch allergen components    |                              | Timothy allergen components      |                                | House dust mite allergen components |                              |                              |  |
|----------------------------------|------------------------------|------------------------------|----------------------------------|--------------------------------|-------------------------------------|------------------------------|------------------------------|--|
|                                  | N = 64                       |                              | N = 43                           |                                | N = 24                              |                              |                              |  |
|                                  | Bet v 1% (Abs.f)<br>(95% CI) | Bet v 2% (Abs.f)<br>(95% CI) | Phl p 1,5b % (Abs.f)<br>(95% CI) | Phl p7,12% (Abs.f)<br>(95% CI) | Der p1% (Abs.f)<br>(95% CI)         | Der p 2% (Abs.f)<br>(95% CI) | Der p10% (Abs.f)<br>(95% CI) |  |
| $<0.35$ kUA/l                    | 9.37 (6)                     | 84.38 (54)                   | 9.30 (4)                         | 81.40 (35)                     | 32.33 (8)                           | 8.33 (2)                     | 91.67 (22)                   |  |
| (0) undetectable                 | 4.4–19.0                     | 73.6–91.3                    | 3.4–22.0                         | 67.4–90.3                      | 18.0–53.3                           | 2.3–25.8                     | 74.2–97.7                    |  |
| 0.35–0.7 kUA/l                   | –                            | –                            | 9.30 (4)                         | –                              | 8.33 (2)                            | 8.33 (2)                     | 8.33 (2)                     |  |
| (1) very low                     | –                            | –                            | 3.4–22.0                         | –                              | 2.3–25.8                            | 2.3–25.8                     | 2.3–25.8                     |  |
| 0.7–3.5 kUA/l                    | 15.62 (10)                   | –                            | 18.6 (8)                         | –                              | 8.33 (2)                            | 8.33 (2)                     | –                            |  |
| (2) low                          | 8.7–26.4                     | –                            | 9.7–32.6                         | –                              | 2.3–25.8                            | 2.3–25.8                     | –                            |  |
| 3.5–17.5 kUA/l                   | 3.13 (2)                     | 9.37 (6)                     | 34.88 (15)                       | 9.30 (4)                       | 8.33 (2)                            | 16.67 (4)                    | –                            |  |
| (3) moderate                     | 0.9–10.7                     | 4.4–19.0                     | 22.4–50.0                        | 3.7–22.0                       | 2.3–25.8                            | 6.7–35.9                     | –                            |  |
| 17.5–50 kUA/l                    | 34.38 (22)                   | 3.13 (2)                     | 16.28 (7)                        | 9.30 (4)                       | 24.0 (6)                            | 33.33 (8)                    | –                            |  |
| (4) high                         | 24.0–47.0                    | 0.9–11.0                     | 8.1–30.0                         | 3.7–22.0                       | 12.0–45.0                           | 18.0–53.3                    | –                            |  |
| 50–100 kUA/l                     | 18.75 (12)                   | 3.13 (2)                     | 11.63 (5)                        | –                              | 8.33 (2)                            | 24.0 (6)                     | –                            |  |
| (5) very high                    | 11.1–30.0                    | 0.9–11.0                     | 5.1–24.5                         | –                              | 2.3–25.8                            | 12.0–45.0                    | –                            |  |
| $>100$                           | 18.75 (12)                   | –                            | –                                | –                              | 8.33 (2)                            | –                            | –                            |  |
| (6) above the limit of detection | 11.1–30.0                    | –                            | –                                | –                              | 2.3–25.8                            | –                            | –                            |  |

had elevated level of specific IgE to a core protein NPC 2 (nDer p2), the second major house dust mite molecular component. There were no patients who had the result of < 0.35 kUA/l; the class 1 level was 8.33%, class 2—8.33%, class 3—16.67%, class 4—33.33%, and class 5—24.0% of cases. No class 6 antibodies were detected. The assay of antibodies to major house dust mite molecular components demonstrated that high-specific antibody level to the cysteine proteinase was prevalent. Also, high and very high antibody levels to the second major allergen component (core protein MPC-2) were detected. Additionally, 8.33% of patients were sensitized to tropomyosin (nDer p10), a minor house dust mite molecule component.

## 4 Conclusion

The identification of sensitization to major and minor allergens allows the selection of an allergen for ASIT in patients with polysensitization or discrepancies in the clinical history data and results of skin testing with allergen extracts. According to the concept of molecular-based allergen diagnostics, the ASIT efficacy is expected to be high when asIgE detected using extracts to the major components are present, while antibodies to minor components are absent. With simultaneously elevated IgE levels to major and minor ASIT proteins, a moderate ASIT efficacy level is anticipated.

Our data obtained indicate that the IgE asIgE to allergen component level should be taken into account when evaluating the efficacy of ASIT. The maximum ASIT efficacy is expected with a very high level of antibodies (level 5, 50–100 kUA/l) and when level of antibodies is above the detection (level 6, > 100 kUA/l). According to this concept, the best ASIT results will be achieved when level of the major antibodies to the major birch pollen molecular component (rBet v 1) and the major allergen component of house dust mites (core protein NPC 2) are increased, while the levels of moderate and

minor elevation of antibodies to minor antigen components are present. In conclusion, we determined and the predicted the effectiveness of ASIT in patients with polysensitization. More study is required to develop the personalized criteria to evaluate the effectiveness of ASIT.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflicts of interest.

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