A Real Life Comparison between Allergenic Extracts and Allergenic Molecules

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ABSTRACT

Serum IgE assay is a mainstay step in the allergy work up. Allergenic extracts and molecular components are available at present. This real life study compared the serum specific IgE levels against allergenic extracts with allergenic molecules in patients allergic to Parietaria, Betulaceae, and mites.

This retrospective real life study included 489 subjects with respiratory allergy. Inclusion criteria were 1) documented diagnosis of allergic rhinitis (AR) and/or allergic asthma, and 2) documented allergy to Parietaria judaica (Par j 2 (216 patients: 112 females, mean age 42 years), or to Betula verrucosa (Bet v) 1 (62 patients: 35 females, mean age 3 years), or Dermatophagoides pteronyssinus (Der p) 1 (211 patients: 107 females, mean age 34 years); and mono-allergy. Serum IgE, specific both for total/crude allergen extracts and individual purified/recombinant allergens, were assessed by ImmunoCap system.

The serum IgE levels to birch extract were very strongly (R²=0.96) related to IgE to Bet v 1. There was a strong (R²=0.71) correlation between Dermatophagoides pteronyssinus IgE and Der p 1. A very strong (R²=0.87) correlation also existed between Parietaria extract IgE and Par j 2 IgE levels. However, there was discrepancy between percentages of positivity between allergenic extracts and molecules.

Therefore, allergen molecular diagnostics may represent a useful way in allergy work up, but deserves caution in particular circumstances.

Keywords: Allergic rhinitis; Asthma; Bet v 1; Der p 1; Extract; Molecule; Par j 2; Serum IgE

INTRODUCTION

Allergic rhinitis (AR) and asthma have a relevant impact on patients, as they may negatively affect the school and job performances, the daily activities, and the quality of life; the reason of this impressive effect is reinforced by their high prevalence: up to 40% of the general population.1,2

The hallmark of the immune response in patients suffering from type 1 allergy is the on-going production of allergen-specific IgE: the sensitization. Sensitization can be considered the condicio sine qua non (i.e. the
necessary condition) for defining allergy. Poly-sensitization, such as sensitization to more allergens, is an immunological event that is relevant from an epidemiological and clinical point of view. The poly-sensitization prevalence ranges may be up to 90%, with a great variability depending on the investigated population. Nevertheless, a fundamental concept has to be pointed out: sensitization does not always correspond to true allergy. For example, IgE reactivity to cross-reactive carbohydrate determinants (CCD) affects the allergy diagnostic, particularly in pollen allergic patients. Indeed, these substances are responsible for the phenomenon of cross-reactivity of sera towards a wide range of allergens from plants and insects. Positivity to CCDs usually does not elicit clinical symptoms, so CCD sensitization should be regarded as false positivity.

Actually, the allergy diagnosis is based on the demonstration of a consistency between sensitization and the history of symptoms appearance after the exposure to sensitizing allergen.

Sensitization can be demonstrated in vivo [by skin prick test (SPT)] or in vitro [by serum IgE measurement]. Traditionally, natural allergen extracts from allergenic sources are used in the common practice. However, the extract mixtures are usually heterogeneous because they may include not only the major allergens, but also cross-reactive allergens, non-allergenic antigens and interfering substances. To remedy this bias, molecular-based allergy diagnostic tests have been recently introduced in the clinical practice, allowing defining and characterizing exactly the sensitization profile. This methodology is based on allergen molecules that are involved in the specific immune response to allergens. Allergen molecules may be purified, recombinant, or synthetic. The use of molecular allergology has changed the allergy workup, being highly useful in allergen-specific immunotherapy prescription. In fact; the positivity to major allergens excludes false reactivity to pan-allergens. In other words, a patient may be sensitized to many allergens, but he/she may be allergic only to few ones or even to none. On the other hand, the major drawbacks of using only major individual allergens in the diagnosis of allergy may be the lack of other major and minor allergens, which could sensitize some allergic individuals as well as the poor efficiency of some recombinant allergens.

The most common allergies in the area of Liguria region (in the Northwest Italy) are due to pollens, mainly *Parietaria*, *Betulaceae*, and mites, especially *Dermatophagoides*. In this regards, *Parietaria* recombinant allergen (*Par* j 2), *Betulaceae* recombinant allergen (*Bet* v 1), and *Dermatophagoides* allergen (*Der* p 1) are the major and genuine allergens. Therefore, in this study we compared the serum IgE levels against extract allergens and allergenic molecules in patients allergic to *Parietaria*, *Betulaceae*, and mites.

**MATERIALS AND METHODS**

This retrospective real life study included 489 subjects, who referred to the Allergy Department for respiratory allergy in 2014. Inclusion criteria were: 1) documented diagnosis of AR and/or allergic asthma, 2) positivity to allergenic molecules as *Par* j 2 (216 patients: 112 females, mean age 42 years) or *Bet* v 1 (62 patients: 35 females, mean age 53 years) or *Der* p 1 (211 patients: 107 females, mean age 34 years); 3) mono-allergy. Positivity was defined by values>0.35 kU/L. The diagnosis of allergy was based on the consistency between positive SPT and occurrence of nasal symptoms after exposure to sensitizing allergen.

The study was performed according to the Review Board rules and patients gave an informed written consent.

**Serum IgE Assay**

Serum levels of specific IgE were detected by the immunofluorometric assay (IFMA, ImmunoCAP, Thermo Fisher Scientific, Uppsala, Sweden) in peripheral blood samples from patients. Serum was collected into gel-separator tubes, centrifuged and stored at -20°C until analysis. The following allergens were considered: T3 (*Betulaceae* native allergen), and *Bet* v 1; W21 (*Parietaria* native allergen) and Parj2; D1 (*Dermatophagoides* native allergen) and *Der* p1.

Measurement of circulating specific IgE antibodies was performed according to manufacturer’s instructions. Specific IgE concentrations were expressed in kU/L according to the traceable calibration to the 2nd Implementation Research Platform (IRP) WHO for Human IgE and 0.35 kU/L has been considered as a cut-off.

**Statistical Analysis**

Medians (md) and percentiles [25th and 75th],
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Interquartile Range (IQR) were used as descriptive statistics. The non-parametric Mann-Whitney test was used to compare samples. In addition, correlations were calculated by Spearman test. A $p$ value, 0.05 was considered statistically significant. All data were analyzed using the Stata statistical package, Release 13.1 Statistical Software (StataCorp, College Station, TX, USA).

**RESULTS**

**Distribution of Sensitization Frequency**

Basically, there were three possibilities: double positivity, positivity only to extract, and positivity only to molecule as reported in Table 1. About birch, 52 (91\%) patients were positive to both allergens, 5 (9\%) only to birch, and 5 only to Bet v 1. About mites, 151 (71\%) were positive to both allergens, 60 (29\%) were positive only to *Dermatophagoides pteronyssinus*, and nobody was sensitized only to Der p 1. About *Parietaria*, 187 (86.5\%) were positive to both allergens, 28 (13\%) were positive only to *Parietaria*, and 1 (0.5\%) only to Par j 2.

**Serum IgE Levels**

About birch allergy, there was no significant difference ($p=0.12$) between serum IgE levels of the allergen birch extract (md=8.1 kUA/L; IQR=1.7-24.3 kUA/L) and the molecule Bet v 1 (md= 8.0 kUA/L; IQR= 2.2-24.6 kUA/L), as reported in Figure 1. About mite allergy, there was a significant difference ($p<0.0001$) between *Dermatophagoides pteronyssinus* extract (md=15.4 kUA/L; IQR=6.2-51.5 kUA/L) and Der p 1 levels (md 5.6 kUA/L; IQR=1.9-21.0 kUA/L).

In Parietaria allergic patients, there was a significant difference ($p<0.0001$) between *Parietaria* extract (md 15.2 kUA/L; IQR= 5.1-36.8 kUA/L) and Par j 2 levels (md 11.3 kUA/L; IQR=3.9-31.1 kUA/L).

Table 1. Distribution of possible sensitization patterns against allergen extract and allergen molecules: double positivity, positivity only to extract, positivity only to molecule. Numbers and percentages of patients are reported.

<table>
<thead>
<tr>
<th></th>
<th>D1+ Derp1+</th>
<th>D1+ Derp1-</th>
<th>D1-Derpi+</th>
<th>W21+ rParj2+</th>
<th>W21+ rParj2-</th>
<th>W21- rParj2+</th>
<th>T3+ rBetv1+</th>
<th>T3+ rBetv1-</th>
<th>T3-rBetv1+</th>
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<td>%</td>
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<td>0</td>
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<td>13</td>
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<td>91</td>
<td>9</td>
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<tr>
<td>n.</td>
<td>151</td>
<td>60</td>
<td>0</td>
<td>187</td>
<td>28</td>
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<td>52</td>
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<td>5</td>
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<tr>
<td>TOTAL</td>
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<td>216</td>
<td>62</td>
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D1=Dermatophagoides pteronyssinus extract; Der p 1=Dermatophagoides pteronyssinus 1 molecule; W21=Parietaria Judaica extract; rPar j 2=recombinant Parietaria Judaica 2 molecule; T3=birch extract; rBet v 1=recombinant Betula verrucosae1 molecule

Figure 1. Comparison between specific IgE levels against allergen extract and allergen molecules: in birch allergic patients (upper left quadrant), in mite allergic patients (upper right quadrant), in Parietaria allergic patients (lower quadrant)
Figure 2. Correlations between specific IgE levels against allergen extract and allergen molecules: in birch allergic patients (upper left quadrant), in mite allergic patients (upper right quadrant), in Parietaria allergic patients (lower quadrant).

Correlations between Extract and Molecule Serum Levels

The serum IgE levels to birch extract were very strongly (r=0.96) related to IgE to Bet v 1, as reported in Figure 2. There was a strong (r=0.71) relationship between *Dermatophagoides pteronyssinus* IgE and Der p 1. A very strong (r=0.87) correlation existed between Parietaria extract IgE and Par j 2 IgE levels.

**DISCUSSION**

Allergen-specific IgE immunoassay is a reliable, standardized analytical method offering a better accuracy than SPT in recognizing allergic patients. In addition, the recent commercial availability of new molecular tests allows a more accurate diagnosis, focused on the recognition of the true causative allergen; this property leads to a significant increase of test specificity because of the exclusion of false sensitization due to cross-reactivity. However, in-vitro molecular allergy tests are expensive and they cannot be performed by every clinical laboratory.

Despite molecular allergy tests which are opening encouraging perspectives for the improvement of patient care, results should be evaluated with caution, taking into account the presence and the severity of clinical signs, since sensitization does not necessarily correspond to true allergy.

On the basis of these assumptions, the present study aimed to compare conventional allergen-specific IgE immunoassay with molecular allergy tests, for the diagnosis of the most important allergies in our area, such as birch, *Parietaria*, and mite allergy.

Bet v 1 is recognized by 91% of birch-positive patients: this finding is relevant as it is consistent with Northern Europe data, but is conflicting with other Italian reports. The possible explanation of this surprising finding might depend on the local imprinting of primary sensitization to the main *Betulaceae* plant present in our geographic area: the hop-hornbeam. Hop-hornbeam has relevant homology with birch.

Conversely, only 9% of birch-positive patients were negative for Bet v 1. Interestingly, 9% of patients were positive only to Bet v 1. A very strong relationship between serum sIgE reactivity to birch extract and Bet v 1 as well as no significant difference between values confirm this outcome. Therefore, it seems to be reasonable to suggest the use of Bet v 1 assessment in
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the clinical practice instead of the birch extract, mainly concerning the Allergy Immunotherapy (AIT) prescription. In fact, about 10% of patients were sensitized only to Bet v 1 and without assessing it, they will not advised for AIT.

About Par j 2, only 13% of Parietaria allergic patients were negative to Par j 2 and there was a very strong relationship between extract and allergen molecules IgE, even though the IgE levels were higher for extract IgE than Par j 2 IgE. In addition, only 0.5% of patients were sensitized only to Par j 2.

There was a high rate (28%) of mite allergic patients who were negative for Der p 1 and nobody was positive to Der p 1 only. Despite a strong correlation between Dermatophagoides pteronyssinus IgE and Der p 1 IgE, there was a significant difference between extract IgE and Der p 1 specific IgE values. This finding is partially in agreement with a recent study about molecular diagnostics in mite allergy.20

On the basis of these findings, we focused our attention on patients sensitized to extract allergens alone: 5 patients for birch, 60 patients for Dermatophagoides p, and 28 patients for Parietaria. We found that most of them were polyclonally sensitized and frequently they were not allergic to the considered allergens.

It is to note that some allergies (such as HDM allergy) could be underdiagnosed by using only purified/recombinant major allergens: in fact, about 30% of D1-positive patients were negative to Der p 1. Regarding this finding, it should be emphasized that a thorough history should be considered in these patients.

Therefore, the present findings show that allergy molecular diagnostics should be appropriately interpreted by allergists, and results should be adequately interpreted. In fact, patients should be carefully evaluated considering the consistency between sensitization and the symptom occurrence after exposure to the sensitizing allergen. The main limitation of this study is that it was retrospective and it was also conducted on a relatively limited number of patients. Another drawback concerns the immunoreactivity comparison between total extracts (T3, W21 and D1) and recombinant forms, the ideal way to clarify the immunoreactivity difference is to perform a western blotting on total extracts or to measure specific IgE to purified native molecules. However, the present study was conducted in real life, providing a direct comparison between “traditional” and molecular diagnostics in the common practice.

Allergen molecular diagnostics should be considered in selected patients by allergy specialists as a complementary diagnostic tool to determine specific sensitization profiles and to predict possible cross-reactivity between different allergenic sources.

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