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Component-resolved diagnosis: Performance of specific IgE to *Alternaria* compared to Alt a 1

To the Editor,

Component-resolved diagnosis (CRD) is able to identify specific IgE (sIgE) to species-specific allergenic molecules and differentiate true sensitization from cross-reaction. This has proved useful in patients diagnosed with sensitization to multiple pollens (1). The correct identification of true sensitization allows the proper choice of allergens for allergen-specific immunotherapy (AIT) and avoids the use of unnecessary allergens.

Likewise, in food allergy, CRD is able to identify patients sensitized to molecules associated to severe reactions, such as lipid-transfer or storage proteins, or to those, such as profilin or PR-10 proteins, associated to mild or no reactions (2). Thus, the use of CRD can change diagnostic and therapeutic approaches.

As more allergens become available for CRD, it is important to evaluate whether they add a benefit to existing techniques. In the case of mold allergy, there is scarce information about their role (3–5). We aimed to compare the *in vitro* performance and the cost utility of sIgE to *Alternaria* and to Alt a 1, its major allergen.

We retrospectively collected the data on sIgE to *Alternaria* and Alt a 1 in serum samples of children visited in our clinics for symptoms of asthma and/or rhinoconjunctivitis and who had positive skin prick tests (SPT) to *Alternaria*, as part of routine clinical practice. Serum sIgE was measured simultaneously following instructions of the manufacturer (ImmunoCAP®; Thermo Fisher Scientific Inc, Waltham, MA, USA). Values <0.35 kUA/l were considered negative; those >100 were

evaluated as 101. The data were analyzed with the software program SPSS 15.0 (IBM-SPSS, Chicago, Ill, USA).

The sample included 255 children (175 M/80 F) aged 2.4–16.1 yr. Negative sIgE was found in 15.3% of cases for *Alternaria*, in 18.4% for Alt a 1 ($p = 0.34$), and in 14.5% for both.

There was a very good correlation between the two determinations ($r = 0.943$, $p < 0.001$) as shown in Fig. 1. Overall, the levels were higher, not significantly, for Alt a 1 (19.01 ± 24.20 kUA/l) than for *Alternaria* (14.73 ± 19.10 kUA/l), $p = 0.42$. There were 232 patients with differences >10% between both values; 165 (71.1%) with higher Alt a 1; 67 (28.9%) with higher *Alternaria*. Table 1 shows the results of the two patients with negative *Alternaria* and positive Alt a 1, and of the ten patients with negative Alt a 1 and positive *Alternaria*.

Component-resolved diagnosis permits a more accurate diagnosis of sensitization with potential impact on therapeutic approaches in the case of pollen, food, or even animal dander allergy (1, 2, 6–8). There is, however, scarce information on the potential impact of CRD on mold allergy (3–5).

Most of our patients were sensitized to Alt a 1, and levels of specific IgE to this allergen were higher than to *Alternaria* (Fig. 1). On the other hand, there were some patients, with long distances plotted under the diagonal, who had much higher values with *Alternaria* than with Alt a 1, meaning that sensitization to other allergens might be relevant.

Overall, the number of positive cases was higher, not significantly, with *Alternaria* (84.7% vs. 81.6%), so this would

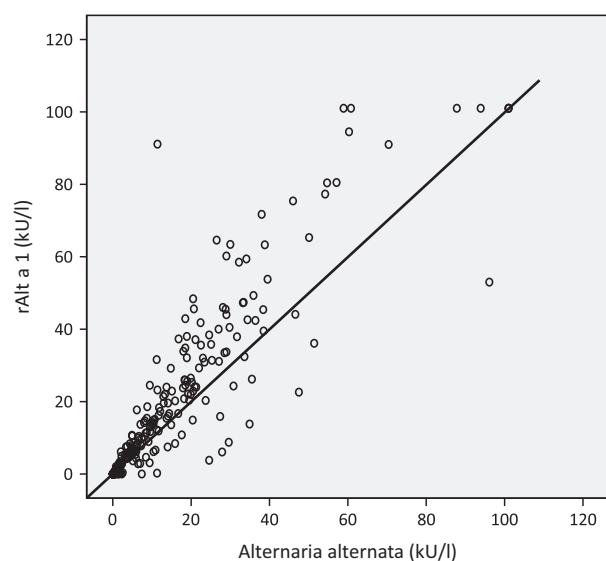


Figure 1 Plotting of results of specific IgE to Alt a 1 and to *Alternaria*. Values above the diagonal had higher results for Alt a 1 than for *Alternaria*, and vice versa for those under the diagonal; the longer the distance to the diagonal the bigger the difference between their values.

Table 1 Values of specific IgE for patients who had positive results for one of the allergens and negative for the other

<i>Alternaria</i>	Alt a 1
Negative <i>Alternaria</i> and positive Alt a 1	
0.23	0.35
0.30	0.39
Positive <i>Alternaria</i> and negative Alt a 1	
0.42	0.02
0.43	0
0.51	0.01
0.56	0.01
1.24	0.01
1.52	0.01
1.96	0.3
2.3	0.2
7.4	0.01
11.3	0.25

allow a better identification of sensitization in an additional 3.1% of patients. If we had determined only *Alternaria*, we would have missed two patients (0.8%) who had positive Alt a 1 (Table 1). However, the values for these were minimally positive, so it does not seem of clinical relevance. The cost of determining sIgE for Alt a 1 in 100 patients is currently €345 more costly than for *Alternaria*, so using *Alternaria* would be a better option if solely for the labeling of patients as sensitized. This would be the option in settings where SPT are not performed, but it would be questionable whether sIgE to *Alternaria* would add relevant information to that provided by SPT.

Current AIT for *Alternaria* is standardized either biologically or by mass units. Biologic standardization does not

provide information on the proportion of the different allergens. Standardization by mass units is more reliable regarding the allergen used. Current AIT formulations in Spain standardizing by this method use only Alt a 1 for this purpose; the rest of *Alternaria* allergens can be thus very variable from batch to batch. Scientific societies and regulatory agencies recommend using only well-standardized products, with reproducible content of allergens (9).

Determining only *Alternaria* leads to the identification as sensitized of a proportion of 3.1% of patients who are negative for Alt a 1 (Table 1). If we use this determination to decide prescription of AIT, we might be prescribing vaccines with Alt a 1 as the only reliable allergen for patients who are sensitized to other allergens. We should not only expect no improvement in this case but also consider the risk of inducing sensitization to other allergens. The current price in our country is €285–610 per year of treatment for subcutaneous and €624–1330 for sublingual AIT formulations, in addition to other non-direct costs. Thus, the savings of not prescribing inadequate AIT in just one of 100 patients clearly exceed the aforementioned difference of €345, and determination of sIgE to Alt a 1 would be a better option, especially considering a recommended minimal 3 yr duration of treatment.

Our study has some limitations, as it aimed to compare only the results of the two *in vitro* tests, but not to assess the concordance with SPT or the relation of tests to type and severity of symptoms. The results of SPT to *Alternaria* were positive in all our patients, but around 15% of them had negative sIgE. This lack of concordance is a well-known fact for the majority of allergens (10) and adds uncertainty about the prescription of AIT in these cases. Many aspects should be taken into account for prescribing AIT, such as severity of symptoms, evolution, response to treatment, sensitizations to other allergens, or others. Age under five is considered a relative contraindication for AIT (9); all the patients in our study who had discordant results for sIgE were older than seven; thus, potential candidates for AIT. *Alternaria* allergens other than Alt a 1, such as Alt a 6, Alt a 9, or others might be important in a certain proportion of patients. The identification of sIgE to these allergens is not routinely available, and immunotherapy products with standardization of them would be needed to make this identification useful.

In summary, the determination of sIgE to *Alternaria* is superior to that of Alt a 1 for a more accurate diagnosis of sensitization and is less costly, but the determination of sIgE to Alt a 1 should be recommended when using the results for the right prescription of AIT and might be cost-effective.

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Conflict of interests

Antonio Nieto and Angel Mazon have participated in clinical trials promoted by Thermo Fisher Scientific and as invited speakers in meetings sponsored by Thermo Fisher Scientific (Formerly Pharmacia and Phadia). The rest of the authors have no conflict of interests.

Author contributions

Maria Nieto contributed to acquisition of data, revision of content. Isabel Lafuente contributed to acquisition of data, revision of content. Rafael Calderon contributed to conception and design, revision of content. Sonia Uixera contributed to conception and design, revision of content. Raquel Pina contributed to acquisition of data, revision of content. Susana Calaforra contributed to acquisition of data, revision of content. Isidoro Cortell contributed to conception and design, revision of content. Antonio Nieto contributed to conception and design, drafting and revision of the

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Vitamin D, virus etiology, and atopy in first-time wheezing children in Finland

To the Editor,
Vitamin D may be protective against acute respiratory infections and atopic illnesses, but studies have been conflicting (1, 2). One likely reason is the vast variability in measurement of vitamin D (based on interview about dietary intake vs. actual measurement of 25-hydroxyvitamin D [25OHD] concentrations) and major differences in study populations (age, confirmation of atopy status, use of vitamin D supplements, and latitude of study region). The recommended minimum level of serum 25OHD varies from 50 to 75 nmol/l (2, 3). In Finland, all young children are instructed to have vitamin D

supplement of 10 µg (400 IU) per day. Despite the recommendations, the 25OHD levels in serum have been deficient (<50 nmol/l) in up to 21–31% of Finnish children (1, 2, 4). In other Westernized populations, 10–17% of the children have been reported to have 25OHD levels <50 nmol/l (2). However, data remain scarce on the 25OHD levels in high-risk children susceptible to viral infections and asthma development, such as young wheezing children (1). To investigate this issue, we analyzed serum 25OHD levels in a cohort of first-time wheezing children that were well characterized for virus etiology and atopy status.

Serum samples and nasopharyngeal aspirates were collected from 107 first-time wheezing children in the Turku University Hospital (Turku, Finland) from June, 2007 to March, 2010. The children participated in a randomized controlled trial, which studied the effectiveness of oral corticosteroid (5). The inclusion criteria consisted of age 3–23 months, delivery at ≥36 gestational weeks, first wheezy episode (parental report and confirmed from

Abbreviations:

B-Eos, Blood eosinophil count; CI, 95% confidence intervals; IgE, Immunoglobulin E; IQR, Interquartile range; PCR, Polymerase chain reaction; RSV, Respiratory syncytial virus; s.d., Standard deviation; 25OHD, 25-hydroxyvitamin D; 25OHD2, 25-hydroxyvitamin D2; 25OHD3, 25-hydroxyvitamin D3.