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Review

Component-resolved diagnosis in allergic disease: Utility and limitations

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ABSTRACT

Component resolved diagnosis (CRD) is a microarray-based diagnostic solution capable of simultaneously analysing specific IgE antibodies against 112 allergenic components, providing sensitivity patterns for multi-sensitised or complex patients. The CRD is indicated for these patients, especially those with concomitant respiratory and food allergies. This study reivews the method, its utility, limitations, and our experience in allergic diseases with difficult etiologic diagnosis (eosinophilic esophagitis, occupational asthma and drug allergy).

1. Introduction

The introduction of microarray techniques has been a major advance in the diagnosis of allergic diseases.

Briefly, this technique, also known as component resolved diagnosis (CRD), shows the real sensitisation pattern of multi-sensitised patients, helps to differentiate between cross-reactivity and co-sensitisation, and helps to rule out allergy [1, 2].

CRD reveals unexpected sensitisations and provides more information on patients with idiopathic anaphylaxis [3–6]. This technique helps to anticipate the risk and the type of reaction (asymptomatic, local or systemic) and therefore guide decisions about food challenges [7–14].

Allergic drug reactions (ADR) are the third most common reason for consultation in allergy services. Globally, ADR affect 10–20% of hospitalized patients and up to 7% of outpatients. However, this might be an underestimate due to underreporting, or an overestimate due to unexplained reactions often being classified as “allergic”. This carries risks for the patient. CRD also provides specific information on penicillin and other drug allergies.

In terms of treatment, CRD helps to identify the IgE profile in patients who are unresponsive to specific immunotherapy [15–18]. Nevertheless, the limitations of these tests have also been described [19].

In this review we describe the methods of this technique and their applications in the diagnosis of diseases in which an allergic mechanism is still controversial.

2. Material and methods

2.1. CRD technique

The Immuno Solid-Phase Allergen Chip ISAC® (Thermofisher Diagnostic, Sweden) is a microarray-based diagnostic solution capable of simultaneously analysing specific IgE antibodies against 112 allergenic components, providing sensitivity patterns for multi-sensitised or complex patients. The CRD is indicated for these patients, especially those with concomitant respiratory and food allergies [14].

CRD can also be useful in the diagnosis of idiopathic anaphylaxis and the identification of major sensitisation patterns in multi-sensitised patients. In multi-sensitised patients, it has been shown that ImmunoCAP ISAC® provides useful and detailed information [1–10].

CRD can obtain 112 results in each test using a small sample volume

Abbreviations: CRD, Component resolved diagnosis; ISU, ISAC standardised units; MIA, microarray image analysis software; IgE, Immunoglobulin E; AIT, Allergen-specific immunotherapy; OIT, oral immunotherapy with food; Api m1, major allergen from *Apis mellifera* venom; Ves v1 and Ves v5, mayor allergens from *vespa* and *vespula* venom; Pol d5, mayor allergen from *Polistes dominulus* venom; EoE, eosinophilic esophagitis; Tri a 14, Lipid transfer protein from wheat; Prup 3, Lipid transfer protein from peach.; SPT, skin prick-test; BA, Baker's asthma

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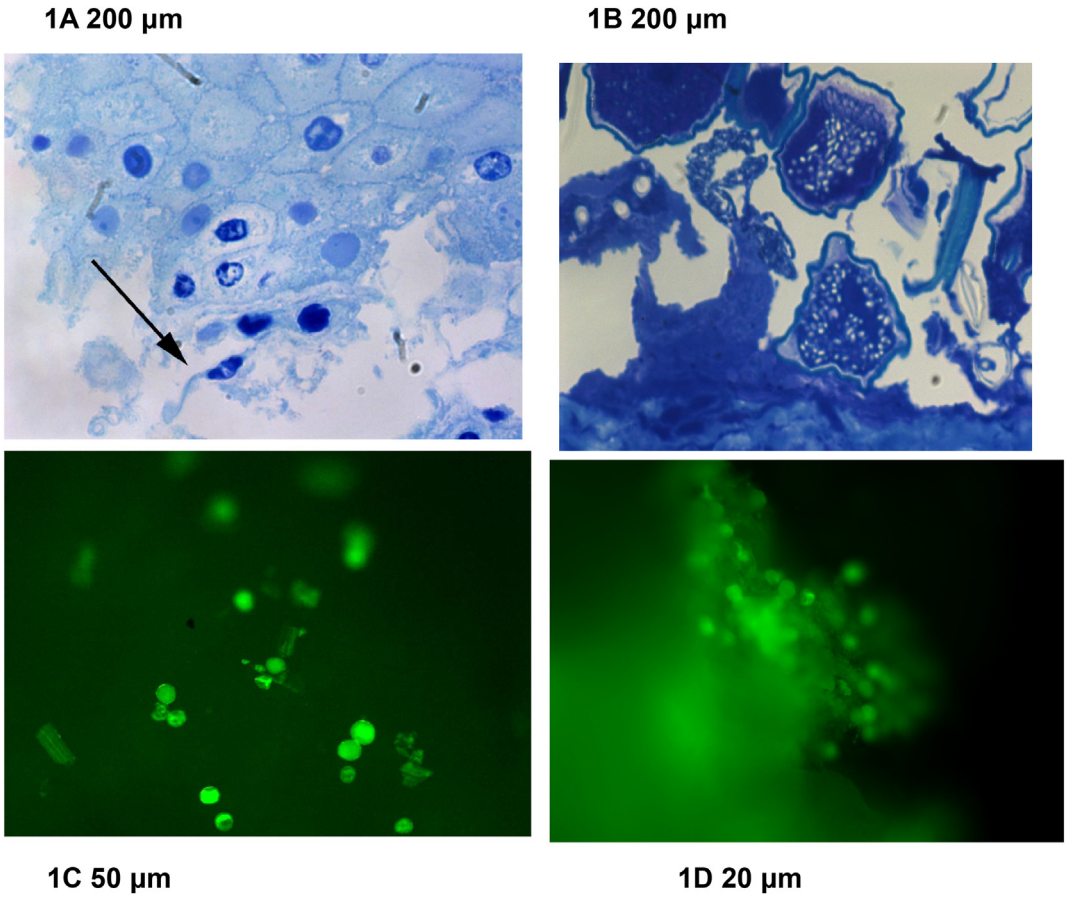

Fig. 1. Epifluorescence (A and B) shows pollen, spores and other plant elements on the surface of oesophageal biopsies before histological fixation. (C and D): Plant impactions and oesophageal mucosae showing damaged epithelial spinous cells. Semi-thin sections with toluidine blue stain. Arrow shows pollen tubes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(30 µl). This panel includes specific species and cross-reactivity related molecules.

Thanks to the cross-reactivity components, in addition to 51 allergenic sources, CRD provides information on hundreds of allergenic sources, like food (milk, eggs, fish and shrimp, nuts, legumes, wheat, fruits), pollen (from weeds, trees, herbs), animals, moulds, mites and cockroaches, parasites, latex and carbohydrates [3].

The broad panel of molecular allergens consists of 112 allergenic components fixed in a biochip. The software generates reports, including orientating remarks to aid interpretation. Only 30 µl of serum or plasma are necessary. The semi-quantitative results are shown in ISAC standardised units (ISU).

Low background noise provides blank results for healthy, non-atopic controls, as well as very good specificity for patients with high total IgE, as is the case in patients with atopic dermatitis. The measurement interval, from 0.3 to 100 ISU, provides information on the IgE antibody levels. The ISUs are standardised according to specific ImmunoCAP units.

Sensitivity varies from 0.3 to 1.0 ISU, depending on the allergenic component. There is no interference, even with a very high total IgE. The allergenic components are deposited in triplets and fixed in a covalent on a polymer-coated microscope slide. Each microscope slide contains 4 microarrays that give results from 4 different samples. The ImnunoCAPis a two-step assay:

1. The IgE antibodies of the patient's serum are combined with the fixed allergenic components.
2. The IgE antibodies joined to allergens are detected through a fluorescent-marked anti-IgE antibody.

The test procedures (including washing and incubation steps) take a total assay time of < 4 h.

Fluorescence is measured with a laser scanner and the results are evaluated using microarray image analysis (MIA) software, which in turn generates a personalised result report.

In summary, the allergen microarray immunoassay ISAC 112 is a repeatable and reproducible in vitro diagnostic tool for determination a specific IgE [14].

2.2. Utility and limitations

CRD enables testing for a specific IgE against multiple allergen components, more than can be tested by prick testing, but some patients present diagnostic difficulty. Results from some studies indicate that CRD may offer increased specificity, but sensitivity is lacking when compared with standard skin-prick testing and measurement of serum food specific IgE levels [12]. Nevertheless, the CRD detects more allergens than prick testing and specific IgE, and can be used when these diagnostic tests do not yield results. So, CRD can be very useful in anaphylaxis caused by hidden allergens [10].

The time taken to carry out the CRD is still too long and the result interpretation depends on qualitative interpretation. Because it is a manual procedure requiring a lot of attention there exists the possibility of human error. The flexibility of the number and types of proteins that can be printed on the microarray allows different sets of specific IgE immunoassay analyses to be carried out [9].

2.2.1. Utility for food allergy

A model combining CRD with clinical background and extract-based

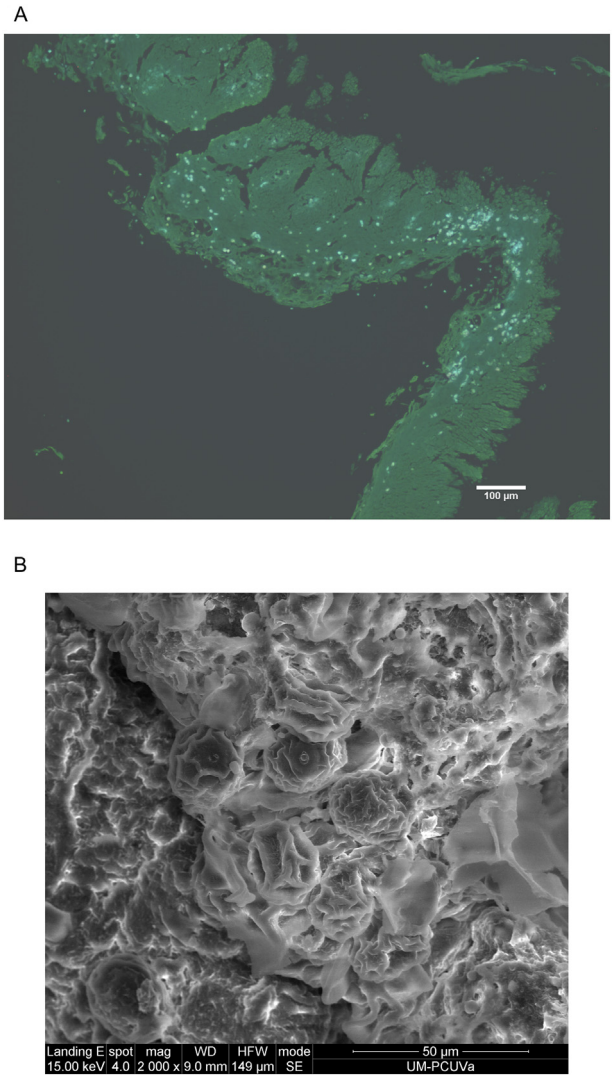

Fig. 2. A: damaged epithelial cells and intercellular spaces: Micro-impaction mainly composed of pollen grains of the *Poaceae* family infiltrating intercellular spaces. Semi-thin sections with using Sirofluor (aniline blue fluorochrome) stain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

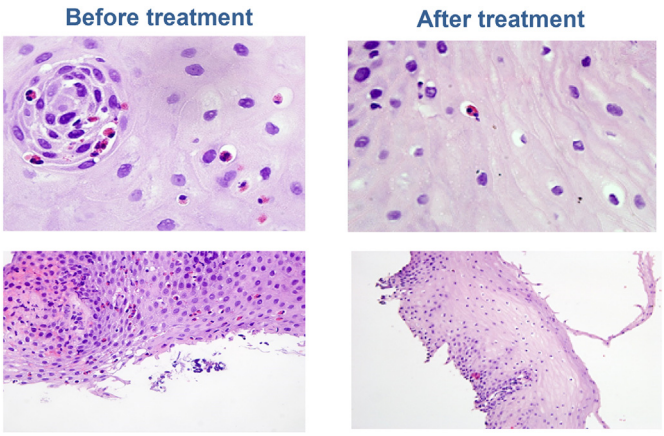

Fig. 3. Human histology showed eosinophilic infiltration before AIT and elimination diet with significant decrease of eosinophil infiltrate at two years. EoE biopsies showed eosinophilic infiltration gradually lessened after etiological treatment with diet and specific AIT. (Before AIT H/E 100×, > 15 Eo/CGA:After AIT (H/E 40×). *H/E: Hematosiline-eosine stain.*

serology is superior to CRD alone in assessing the risk of severe reactions to hidden allergens in foods, particular for ruling out severe reactions (5, 10). CRD provides information on many allergens, but some important different allergens, like tomatoes, fruits, parasite allergens, drugs, occupational allergens and others, are still not included in the microarray commercialised by Thermofisher®. In some studies, the ImmunoCAP test should be the preferred single test for possible allergy to nuts, wheat or other specific foods. In these conditions, SPT and ISAC tests give comparable results. For the authors of this paper, the most useful single test for oral allergy syndrome is ISAC, and SPT should be the preferred test for latex allergy [7].

2.2.2. Utility in hymenoptera hypersensitivity

There is also the possibility of false negative and positive results using ISAC. This is of special importance in the diagnosis of hymenoptera hypersensitivity. Although recombinant allergens could also be used by conventional sIgE testing by ImmunoCAP, component-resolved diagnosis based on the use of well-defined, properly characterized and purified natural and recombinant allergens constitutes a new approach in the diagnosis of venom allergy [1]. In recent years, CRD has allowed for the measurement of sIgE antibodies against major allergens Api m 1, Ves v 1, Ves v 5, and Pol d 5, as well as cross-reactive carbohydrate determinants (CCDs). These tests are intended to help determine the clinical relevance of any given sensitisation, especially in patients with dual sensitisation. (Component-resolved tests are a valuable addition to the diagnostic spectrum so long as they are used in combination with established procedures). Apart from Ves v 5, measuring IgE antibodies to Ves v 1 should always be included in the diagnostic workup [6]. Cross-reactive carbohydrate determinants (CCDs) in plants and insect venoms are a common cause of irrelevant positive test results during in vitro allergy diagnosis. Some CCD-positive sera show nonspecific IgE binding even with CCD-free recombinant allergens when using the Phadia ImmunoCAP® platform [3]. The diagnostic gap of previously undetected Hymenoptera allergy has been decreased via production of recombinant allergens. Knowledge of analogous interspecies proteins and cross-reactive carbohydrate determinants is necessary to distinguish relevant from irrelevant sensitizations [4].

2.2.3. Utility in allergy caused by animal and parasites

CRD is useful in the diagnosis of animal allergy [4]. The prevalence of hypersensitivity to marine parasite allergens other than *Anisakis simplex* should be studied, and the most appropriate technique for this may be CRD [8].

2.2.4. Utility in pollen allergy

In pollen allergy, the clinical benefit of CRD in patients sensitised to pollen and fruit-related allergen is very important [13]. The features of the ISAC 112 microarray are similar or superior to those of ImmunoCAP. The CRD is particularly useful for the etiologic diagnosis of pollinosis in patients sensitised to multiple pollen species whose pollination periods overlap [17].

2.2.5. Utility in allergen challenge test

Another important utility of CRD is to control the risk in allergen challenge. Challenge tests for food-dependent, exercise-induced anaphylaxis carry risk, and have a high rate of false negatives [11]. CRD has the potential to provide information on allergen molecules associated with anaphylaxis in order to determine a food challenge and to make assessments of clinical reactivity to food allergens more accurate [12].

2.2.6. Utility in deciding immunotherapy

Some studies show that SPT is less expensive than allergen molecule-based diagnostic testing. However, allergen molecule-based serology is more precise in detecting disease-causing allergen sources and allows a more precise prescription of immunotherapy, which

Table 1
Clinical outcomes of EoE patients after two years AIT and/or elimination diet.

Intervention 129 patients with EOE	No AIT/no avoidance	AIT only	Avoidance only	AIT + avoidance	Pollen/pollen tubes	Callose
AIT	19	23	19	68	80	76
Group 1 grasses pollen		22		33	55	55
Other pollen mixtures		1		25	25	21
Avoidance						
Hazelnut			1			
Hazelnut + walnut			2			
Peach/fruits			16			
AIT/avoidance						
Hazelnut				23		
rCor a8/hazelnut				22		
Peach/fruits				15		
Sea food				8		
Significant improvement at 2 years	1 (5.2%)	22 (95.6%)	14 (73.7%)	64 (94.1%)	7	3
Symptom free at 2 years	1 (5.2%)	22 (95.6%)	11(57.9%)	64 (94.1%)	6	2

substantially reduces treatment costs and combined costs for diagnosis and treatment [2]. In a "real life" study with 118 patients, a lower number of immunotherapy treatments ($n = 119$) was needed for molecular diagnosis, compared to extract-based diagnosis ($n = 275$), which considerably reduced the total costs for diagnosis and a 3-year treatment from €1112.30 to €521.77 per patient [18].

Allergen molecule-based diagnosis has been suggested to facilitate the prescription of allergen-specific immunotherapy (AIT) [2, 15, 16, 18–23]. The potential role of CRD in circumstances such as the indication of AIT, like pollen polysensitization, food allergy, latex allergy or anaphylaxis, needs a structured approach and more clinical trials [16]. Molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area [18].

In summary, according to the consensus document of the world allergy organization [19], CRD visualises the allergic course and molecular spread in the preclinical stages of allergic diseases, detecting unknown sensitisation, and may indicate that the likelihood of developing symptomatic allergy [15] is associated with specific profiles of sensitisation to allergen components. It is also a useful tool in routine allergy diagnostics due to its ability to improve risk assessment and better select relevant allergens for immunotherapy. In this way, the experience of our group in the diagnosis and treatment with AIT of eosinophilic esophagitis, vernal conjunctivitis, occupational asthma and illicit drug hypersensitivity, and the application of a more effective and precise AIT are summarized below.

2.3. Treatment of eosinophilic esophagitis guided by component resolved diagnosis

Eosinophilic esophagitis (EoE) is characterized by oesophageal dysfunction and, histologically, by eosinophilic inflammation. There is no etiologic treatment. Component resolved diagnosis (CRD) with microarrays can detect possible allergens involved and indicate an elimination diet and allergen immunotherapy (AIT).

No treatment modifies the natural history of EoE, and there are no accepted therapeutic targets defining treatment efficacy, which, together with the wide heterogeneity of EoE patients, makes common strategies very difficult.

In a study of 67 EoE patients we found CRD-guided diagnosis and allergen immunotherapy (AIT) showed a high percentage of patients who were sensitised to environmental allergens, especially pollens, and that after three years CRD-guided diet restriction and AIT, EoE significantly improved [20]. Other recent studies have demonstrated a similarity in AIT response with allergic asthma [21] and the relationship with pollen allergy due to the impaction of pollen and introduction of pollen tubes in oesophageal mucosa [22, 23].

We first hypothesized that, as the esophageal and bronchial mucosa share the same embryonic origin [24], they might respond with similar inflammatory mechanisms to environmental and food allergenic stimuli, and that asthma due to allergens and esophagitis may have an equivalent response to AIT.

Some reports suggest that so-called "immunotherapy" with food—in fact, the induction of tolerance or oral immunotherapy, or OIT, not to be confused with AIT—, is not indicated in EoE. Meta-analyses have been based on very few valid studies. Lucendo [25] selected only three of the 118 reports considered due to their methodology, excluding two good studies in which AIT with aeroallergens improved these patients; he concluded that oral immunotherapy was related to EoE in 2.7% of patients, although the endoscopic study before AIT was not clear. In an EoE patient hypersensitive to a food, the induction of tolerance with the same food could present problems, as could any desensitization technique, albeit controlled.

We also hypothesized that the inflammatory response of the oesophageal mucosa in patients with high levels of antibodies to pollen allergens and worsening seasonal EoE may be due to swallowing airborne pollen, and the intrusion into the oesophageal mucosa of pollen tubes emitted after pollen germination encounter a pH and humidity resembling the stigma at pollination [26, 27], which might be facilitated by desmoglein deficit [8]. Histological analysis may show callose from pollen and other plant products in the oesophageal mucosa.

We aimed to fulfil the classical Koch-Henle postulates [28], which show that a causal agent must be present in each case and not be found randomly in other diseases or healthy controls, and can be identified in all damaged tissues.

The objectives of this study were: to obtain an accurate etiological diagnosis of EoE using standard allergy tests and CRD; to demonstrate a pathogenic role for environmental allergens in EoE using human and plant histology; and, to evaluate the effectiveness of CRD-guided specific AIT and/or elimination diet.

We conducted an observational, longitudinal study to compare the effectiveness and safety profile of CRD-guided specific AIT and/or elimination diet with usual EoE maintenance therapy over a 5-year period of real time analysis (real world study). All suitable patients with EoE from two hospitals and 21 primary care centres in the Autonomous Community of Castile and Leon, Spain, were identified from practice databases and invited to participate in the study. Inclusion criteria were a diagnosis of EoE (symptoms of food impaction and $>$ 15 eosinophils/field on endoscopic biopsy), followed by our Gastroenterology Service from 2010, with a proton pump inhibitor (PPI) trial to confirm the diagnosis, and treated for at least nine months with conventional therapy without clinical improvement.

129 patients with EoE were tested for environmental and food allergens. CRD, histological and botanical analyses were performed. Clinical scores and endoscopic biopsies were performed every six months for 3 years.

Fifty healthy patients, 50 asthmatics due to pollen and 53 celiac disease patients were included as comparison groups. CRD-directed AIT was administered in 91 EoE patients and elimination diet in 140 patients (87 EoE and all 53 CD patients).

CRD detected allergen hypersensitivity in 87.6% of patients with EoE. The predominant allergens were grass group 1 (55%), lipid transfer proteins (LTP) of peach and mugwort, hazelnuts and walnuts. Callose from pollen tubes was found in 65.6% of biopsies. (Figs. 1, 2, 3).

After CRD-guided elimination diet and/or AIT, 101 (78.3%) EoE patients showed significant clinical improvement ($p < .017$) and 97 (75.2%) were discharged (negative biopsy, no symptoms, no medication) without relapse. AIT-treated patients had better outcomes (odds ratio 177.3, 95% CI 16.2–1939.0).Table 1.

In conclusion, CRD-directed AIT and/or elimination diet was efficient in treating EoE patients and was well tolerated [21].

2.4. Utility of component resolved diagnosis in occupational asthma

Wheat is a potent allergen source and can cause food allergy and baker's asthma by wheat flour inhalation. [29]. Prevalence of occupational asthma accounts for 10% of all reported cases of asthma. Baker's asthma (BA) is the most prevalent occupational respiratory disease. The correct diagnosis is important due to the health and legal impact of this disease.

During the past years we have purified and characterized different proteins associated with flour allergy, in order to improve the diagnosis with specific bronchial challenges. We previously found that sensitization to wheat LTP appears more important in wheat food allergy than in baker's asthma and up-regulation of LTP in plants, to increase its resistance against pathogens may imply an increased risk for LTP allergic patients and bakers. Specific challenges had important risks and difficult technical requirements, and the next step was to try CRD techniques [30].

In the last study allergen profiles were compiled of patients with BA from 3 different regions in Spain (Madrid, Malaga, and Valladolid) with an important bakery industry. Forty-five bakers with asthma due to occupational exposure to wheat flour, and with a confirmed diagnosis of occupational asthma by positive skin prick-test (SPT) and positive specific bronchial challenge, were selected for CRD study [30]. We found that > 80% of these patients recognized some of the printed allergens. The highest prevalence of IgE-binding was observed for WTAI-CM16 (54% positivity) and Tri a14 (45% positivity).

The wheat allergen profile in our BA population was not influenced by grass pollen or other environmental allergen patterns. Another fact worth mentioning is that Tri a 14 was recognized only by patients with BA (44%), but not by those who were diagnosed with asthma due to other causes. IgE sensitization to Tri d LTP is closely associated with IgE reactivity to Pru p3 from peach.

Specific IgE values to wheat, Tri d LTP, Tri a 14 and Pru p 3 were determined by ImmunoCAP testing. Allergenic potency and cross-reactivity of Tri d LTP, Tri a 14 and Pru p 3 was investigated by in vitro mediator release and IgE competition assays, respectively.

Tri d LTP shares 48% amino acid identity (aa-id) with Tri a 14, but 52% aa-id with Pru p 3. Recombinant Tri d LTP displayed conserved secondary structure comparable to Pru p 3, whereas nsLTP-specific IgE values were lower in patients with BA. Tri d LTP displayed allergenic potency and IgE cross-reactivity with Pru p 3. IgE cross-reactivity between both wheat LTPs varied between individual patients. For the first time an allergen in *T. durum* was identified. Sensitization to Tri d LTP is closely associated to Pru p 3-mediated food allergy and appears to be more important in wheat food allergy than in baker's asthma [31].

2.5. Utility of CRD in hypersensitivity to illicit drug hypersensitivity

Illicit drugs can cause allergic sensitization in some drug abusers and atopic patients. We have used ImmunoCAP and ISAC in patients with response to *Cannabis sativa* and cocaine, diagnosed after positive bronchial challenges [32]. The CRD confirmed positivity to LTPS. These reports suggest that cannabis sensitization may be mediated by 2 mechanisms, cross-reactivity (mainly with LTPs and thaumatin like proteins), and exposure-related de novo sensitisation. LPTS sensitise primarily through the airways. We characterized the molecular sensitisation profile of patients diagnosed with primary cannabis allergy, who experienced asthma after cannabis or cocaine handling or smoking [33, 34].

3. Conclusions

- Molecular analysis or CRD with recombinant and native allergens can be useful in diagnosis of allergy diseases.
- Molecular analysis or CRD with recombinant and native allergens can be performed in diseases in which allergic mechanisms are in doubt like eosinophilic esophagitis, occupational asthma and illicit-drug allergy.
- Serum testing with CRD can be a useful tool to guide specific immunotherapy if the antigen cannot be avoided.
- Molecular microarray analysis was useful in decision of treatment, and allowed us to make a more restricted allergen elimination and more precise determination for specific immunotherapy.

Author contributions

I declare that the authors: Alicia Armentia and Sara Martín have participated in the conception, design of the study, analysis and interpretation of the data. Blanca Martin carried out all laboratory analyses and Sara Martin the study of celiac patients. All authors have participated in the preparation and critical revision of the paper and all authors have seen and approved the final version of the manuscript. I also declare that no authors have any conflict of interest in connection with this paper.

Conflicts of interests

The authors report no conflict of interest.

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