## Lessons Learned From Component-Resolved Diagnosis in Anaphylaxis: Analysis of a Case Series Based on the International Anaphylaxis Registry

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The diagnostic work-up for anaphylaxis aims to identify triggers, minimize the risk of recurrence, provide information for prognosis, and, in some cases of Hymenoptera anaphylaxis and food allergy, enable administration of etiological treatment. Component-resolved diagnosis (CRD) could prove essential if we are to accomplish such goals. CRD is currently applied when prescribing immunotherapy, assessing the risk of food allergy, and evaluating idiopathic anaphylaxis and pollen-food syndromes [1-3]. Proposed uses of CRD in anaphylaxis [2-3] include exposure to multiple foods, cofactor-enhanced food allergy, and anaphylaxis induced by latex, idiopathic causes, Hymenoptera, and red meat. These proposals need to be confirmed with real-world findings [4]. Our aim was to evaluate the usefulness of CRD in a series of cases included by our department between 2012 and 2021 in the International Registry of Anaphylaxis (IAR), which was supported by the Network for Online Registration of Anaphylaxis (NORA) [5]. The IAR consists in standardized data collection using structured online questionnaires including clinical data (demographics, severity, elicitors, cofactors) and allergy work-ups [5]. We also analyzed complete CRD results for our cases.

The determinations included skin prick tests (SPTs) with purified profilin (Pho d 2) (ALK), lipid transfer protein (LTP) (Pru p 3) (Roxall), tropomyosin (Pen m 1) (Leti), ovomucoid (Gal d 1), ovalbumin (Gal d 2), lysozyme (Gal d 4) (Leti), ß-lactalbumin (Bos d 4), ß-lactoglobulin (Bos d 5), and casein (Bos d 8) (Roxall). All SPTs were performed according to the guidelines of the European Academy of Allergy and Clinical Immunology [6]. Specific IgE determinations were performed using a uniplex assay (ImmunoCAP, Thermo Fisher; cut-off, 0.35 kU/L) and/or a multiplex assay (ImmunoCAP ISAC, Thermo Fisher; cut-off, 0.3 ISU) [1]. Tests were ordered on an individual basis, according to the clinician's judgment and current practice.

Cases were classified by the elicitor group according to the IAR. Allergens were divided between causative and cosensitizations based on clinical judgment. The cofactors were distributed according to patients' allergen profiles. The analysis was performed using SPSS 22.0 for Windows (IBM Corp.), BMDP Statistical Software release 7, StatXact (Cytel Software Corp).

The usefulness of CRD was categorized as follows:

a. Not performed: cases without determinations despite the availability of CRD for the suspected biological source (eg, hake anaphylaxis without parvalbumin determinations).
b. Unnecessary: according to the suspected elicitor and the clinician's judgment (eg, amoxicillin anaphylaxis).
c. Unavailable: no CRD available for the suspected biological source at inclusion in the IAR (eg, venom allergens, during the early years of the IAR).
d. Inconclusive: cases with negative results for currently available allergens (eg, idiopathic anaphylaxis, banana anaphylaxis with negative ImmunoCAP ISAC determinations).
e. Diagnostic: findings for culprit allergens, biological source, and clinical picture are both concordant and conclusive.

We included 116 cases. Patients' characteristics (age, severity, presence of cofactors, individual culprit) are detailed in Supplementary files 2 and 3. Elicitor groups included foods (62.1%), drugs (22.4%), Hymenoptera (7.8%), idiopathic causes (3.4%), other culprits (royal jelly and *Anisakis simplex*) (2.6%), and association with simultaneous exposure to different elicitor groups (tick bites plus food intake) in 1.7% of cases.

The CRD determinations included (number of cases [%]) SPT (82 [70.7%]), uniplex assay (ImmunoCAP, 64 [55%]), and multiplex assay (ISAC, 22 [19%]).

The usefulness of CRD varied depending on the elicitor groups and according to the specific culprits (Table). SPTs with purified allergens performed in 27% of cases of drug anaphylaxis were negative and considered inconclusive. CRD was considered unnecessary in other cases of drug anaphylaxis. CRD was diagnostic in 68.1% of cases of food anaphylaxis. Usefulness was maximum when assessing plant food–induced anaphylaxis and simultaneous exposure to multiple food groups. Within the 13 registered cases of allergy to shellfish, only 2 patients tested positive to tropomyosin. These results suggest the need to consider other allergens, such as arginine kinase and sarcoplasmic protein, and other diagnostic tests, to optimize subsequent advice in shellfish allergy [7]. They also point to current limitations in allergen availability regarding this food.

CRD was diagnostic in Hymenoptera anaphylaxis when it became available. CRD was useful in 2 cases involving mixed-group allergens, but insufficient in suspected idiopathic anaphylaxis or cases involving "other elicitors".

Analysis by culprit allergens showed nonspecific LTPs to be the most frequent elicitors in fruits and nuts and after simultaneous exposure to several food groups. Nonspecific LTPs were considered the only cause in 27 cases. Other profiles, including cosensitization and monosensitization to other allergens, were less frequent. A recent reassessment of

**Table.** Results of Component-Resolved Diagnosis.

| Elicitor group (No.) | Food groups | Causative allergens (No. of cases) | Cosensitization | Usefulness of CRD |
|---|---|---|---|---|
| Foods (72) | Fruits (14) | Bromelain (1)<br>GRP (1) LTP (6)<br>LTP + thaumatin (1)<br>NAA (5), NP (0) | Absent (11)<br>Art v 1 (1)<br>LTP, PR10, thaumatin (1)<br>Parvalbumin and tropomyosin (1) | D 64.3%<br>I 35.7%<br>NP 0 |
| | Nuts/Tree nuts (16) | Albumin 2S (1)<br>Albumin 2S + LTP (1)<br>Albumin 2S + G11S (2)<br>LTP (8)<br>NAA (3), NP (1) | Absent (14)<br>Profilin (1) | D 75%<br>I 18.8%<br>NP 6.25% |
| | Vegetables (4) | LTP (3)<br>NAA (1), NP (0) | Absent (2)<br>LTP (1)<br>PR10 (1) | D 75%<br>I 25%<br>NP 0% |
| | Grains (3) | Gliadin (1)<br>LTP (1)<br>NAA (1), NP (0) | Absent (2)<br>Globulin 11 S (1) | D 66.7%<br>I 33.3%<br>NP 0% |
| | Animal foods (20) | Galactose-α-1,3 galactose (2)<br>Casein (2)<br>Ovalbumin and ovomucoid (2)<br>Parvalbumin (1)<br>Sarcoplasmic calcium-binding protein (1)<br>Tropomyosin (2)<br>NAA (8), NP (2) | Absent (14)<br>LTP and profilin (1)<br>Ovalbumin (1)<br>Profilin (2) | D 55%<br>I 35%<br>NP 10% |
| | Legumes (8) | Albumin 2S, globulin 7S and globulin 11S (2)<br>LTP (2)<br>PR10 (1)<br>UA (2), NP (1) | Absent (5)<br>LTP (1)<br>Profilin (1) | D 62.5%<br>I 25.5%<br>NP 12.5% |
| | Seeds (1) | LTP (1) | Absent (1) | D 100% |
| | DFG (6) | Gliadin (1)L<br>LTP (5) | Absent (4)<br>Ovalbumin, Tri a 14 and aA-Ti (1)<br>PR10 (1) | D 100% |
| DEG (2) | | Galactose-α- 1,3 galactose (2) | Ani s 1 (1) | D 100% |
| Idiopathic (4) | | NAA (2), NP (2) | Absent (2) | D 0 % |
| Hymenoptera (9) | | Ves v 1, Ves v 5, and Pol d 5 (2)<br>Ves v 5 and Pol d 5 (1)<br>Api m 1, Api m 2, Api m 3, Api m 5, Api m 10 (1)<br>UA (5) | Absent (2)<br>Api m 5 (1)<br>Ves v 5 and Pol d 5 (1) | D 44.4 %<br>UA 55.6% |
| Other (3) | | NAA (1)<br>UA (1), NP (1) | Absent (1)<br>Parvalbumin (1) | D 0%<br>I 66.7%<br>NP 33.3% |

Abbreviations: CRD, component-resolved diagnosis; D, diagnostic; DEG, simultaneous exposure to different elicitor groups; DFG, different simultaneous food group intake; I, inconclusive; LTP, lipid transfer protein; NP, not performed; NAA, negative results for available allergens; UA, unavailable for suspected source.

a case previously attributed to LTP confirmed sensitization to gibberellin-regulated proteins [1]. Among animal foods, shellfish was the most frequent culprit. Besides tropomyosin, allergens of animal origin included galactose-α-1,3-galactose, milk, and/or egg allergens.

Fewer patients were affected by Hymenoptera, "other elicitors", and mixed-group allergens, with results showing the difference made by the availability of venom allergens and galactose-α-1,3-galactose over the Registry years assessed.

The usefulness of CRD in food allergy has been addressed according to the clinical scenario [3].

We observed high rates of potential cofactors (according to the IAR) in most elicitor groups ($P$=.003; $\chi^2$ test, Supplementary files 1 and 2). Based on the sensitization in the allergen profiles (Supplementary File 1), the highest percentage of cofactors was observed in patients sensitized to galactose-α-1,3-galactose. Cofactors were present in half of all cases of LTP-induced anaphylaxis, although cofactors

 

were also very frequent in cases where culprit allergens were not identified.

Four patients with idiopathic anaphylaxis were included. Assessment of idiopathic anaphylaxis using CRD includes several options. Negative ImmunoCAP ISAC results would exclude sensitization to multiple major allergens, and this in itself would be an important outcome. Negative results might also suggest sensitization to allergens not included in current assays or mechanisms other than IgE-mediated mechanisms. The differential diagnosis of idiopathic anaphylaxis includes exposure to hidden allergens, *Anisakis*, mast cell activation, and α-gal syndromes [8]. These cases were included over 9 years, with important changes in currently available tests, such as Hymenoptera allergens, galactose-α-1,3-galactose (and its association with idiopathic anaphylaxis) [3,8], and, recently, gibberellin-regulated proteins. We wonder whether our findings for idiopathic anaphylaxis would have been the same before these additions. In the "other elicitors" group, usefulness may be hampered by the multiple allergens included in royal jelly [9].

Negative CRD results may be helpful when assessing drug-induced anaphylaxis (eg, food allergy with nonsteroidal anti-inflammatory drugs as a cofactor vs anaphylaxis induced by these drugs), a surmountable obstacle in some cases of food allergy if we have enough clinical data on biological sources. However, they can render diagnosis challenging when complex allergen sources are involved and when evaluating idiopathic anaphylaxis.

The usefulness of CRD in anaphylaxis varies according to the elicitor group and between triggers within the same group. The usefulness of this approach is expected to grow over time, as the description and availability of allergens increases. Cases of idiopathic anaphylaxis persist despite the appearance of new allergens and identification of α-gal syndrome.

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*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

*Previous Presentations*

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