Component Resolved Diagnosis

“diagnóstico desglosado por componentes”.

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Allergic diagnosis

- Diagnosis as well as specific immunotherapy are currently performed with extracts obtained from natural allergen sources (a mixture of allergenic and nonallergenic components).

- Are often cumbersome to standardize

- Positive reactions to a given allergen extract means that an allergic subject is sensitized against extract components, but without identifying them.

- Negative reactions may indicate non-sensitization to components- perhaps non included in this mix-
"Diagnóstico desglosado por componentes”.

- Con alergenos recombinantes o purificados nos revela un perfil de reactividad del anticuerpo de un paciente alérgico e identifica la molécula alergénica que puede provocar la enfermedad y predecir la relevancia de la sensibilización clínica.

- Component-Resolved Diagnosis
  - It has been demonstrated that cocktails of recombinant allergens can be assembled and improve diagnostic sensitivity.
  - CRD will also allow the precise selection for specific immunotherapy to which a patient is actually sensitized and may increase its safety and efficacy.
  - Help to interpret cross-reactivity
Current (or near future) recombinants/purified Phadia catalog

- Pollens: rBet v1, rPhl p 1, 2, 5, 11, rPar j 2, nOle e1
- Polcalcins :rPhl p 7, rBet v 4
- LTPs: rPar j 2, rPru p 3
- Profilin: Bet v 2, Phl p 12
- CCD: MUXF3 (purified glycopeptide from bromelin)
- Danders: rFel d 1, rCan f 1, rCan f 2, cat, dog and horse serum albumins
- Molds: rAsp f 1,2, 3,4, 6, rAlt a 1
- Food: rAra h 8, rGly m 4, α-Lactoalbumin, β-Lactoglobulin, Casein, Ovoalbumin, Ovomucoide, lysozyme, fish parvalbumin
- Tropomyosin: rPen a 1
- Latex: rHev b 1,2,3,5,6,8,9,11
- Bacteria, staph enterotoxin A, B, C, D, TSST

EGG AND MILK ALLERGY

- Another example of how CRD may be useful in clinic is the diagnosis of milk and hen’s egg allergy.
- Since many years ago we dispose of several purified milk and hen allergens that increase the diagnosis sensitivity and may predict clinical tolerance.

- The following purified allergens are available in CAP system: from milk(Bos domesticus) : Bos d 4 alpha-lactalbumin, Bos d 5 beta-lactoglobulin, Bos d 6 serum albumin, Bos d 7 immunoglobulin, Bos d 8 casein
- and from egg hen (Gallus domesticus) Gal d 1 ovomucoid, Gal d 2 ovalbumin, Gal d 3 Ag22, conalbumin, transferrin, Gal d 4 lysozyme, Gal d 5 serum cklcken albumin (major allergen in bird-egg syndrome)
Grass Pollen-Specific Marker Allergens

- Group 1 allergens: IgE in \(\approx 90\%\) of grass pollen-allergic
- Group 2 allergens: in \(\approx 60\%\) of grass pollen-allergic
- Group 2 allergens are not expressed in *Cynodon dactylon*, *Zea mays* and *Phragmites australis*. In contrast to group 1 allergens
- Group 5 allergens in \(\approx 80\%\) (depends on geographical locations) not detected in *Z. mays* and *C. dactylon*.
- Group 6 allergens are recognized by 60–70% of grass pollen-allergic patients and have been detected in only a few grass species (e.g. timothy grass, Kentucky bluegrass).

- Group 1, 2, 5, and 6 are expressed in grass pollens and are absence in other plants or plant products.

Grass Pollen-Specific Marker Allergens

- Diagnostic tests based on recombinant rPhlp 1, rPhl p 2, rPhl p 5 and rPhl p 6 will thus specifically detect patients with a genuine sensitization to grasses
- For example, patients showing rPhl p1 and rPhl p 2, 1-specific IgE but lacking IgE to rPhl p 5, rPhl p 6 are likely sensitized to certain grass or monocot pollens (e.g. *C. dactylon*, *Z. mays*).
- Patients with rPhl p 6-specific IgE are likely sensitized preferentially towards timothy grass or Kentucky bluegrass.
- Patients lacking IgE antibodies against any of the four recombinant marker allergens may appear positive in grass pollen extract-based diagnostic tests because of IgE binding to other grass pollen proteins with cross-reactive potential (profilin, calcium binding protein).
Grass Pollen-Specific Marker Allergens

• Most pollen extracts used for immunotherapy are mainly standardized regarding their contents of group 1, 2, 5 and 6 allergens

• Caution against immunotherapy with grass pollen extracts in patients lacking IgE antibodies against any of the four marker allergens (i.e. rPhl p 1, rPhl p 2, rPhl p 5, rPhl p 6).

• There are now available from timothy grass Phleum p 1, 5, 6, 7, 11, 12

Birch Pollen-Specific Marker Allergens

• Bet v 1 is the major allergen in birch pollen and crossreacts with the major pollen allergens from Fagales trees and a variety of plant food allergens (soy, peanut).

• Patients reacting with Bet v 1 may be suited for immunotherapy with birch pollen extract

• Whereas a lack of specific reactivity to Bet v 1 suggests sensitization to other panallergens (Bet v2, Bet v4 ) and immunotherapy may not be the most appropriate treatment.

• Bet v 1, 2 and 4 are available in CAP system
Recombinants Allergens and pollinosis

• Birch:
  • + Bet v1 can be associated with sensitization to roacea
  • + Bet v2 (profilin) sensitization to umbilferae

Panallergens Specific Marker Allergens Profilin

• Profilins are important components of the plant cytoskeleton

• Because of their highly conserved biological function, profilins from various organisms share sequence homology

• Accordingly, there is extensive cross-reactivity of allergic patients’ IgE antibodies with profilins from different plant sources. (pollens, vegetables food)

• Profilins are well established relevant in tree, grass and weed pollens and are also cross-reactive allergens expressed at lower levels in somatic plant tissues (e.g. fruits, leaves, seeds, roots).
Panallergens Specific Marker Allergens

Profilin

- Pathogenic role as inducer of respiratory symptoms in pollinosis is uncertain.
- Patients producing specific IgE antibodies are either sensitized or at risk of developing allergic reactions to various plant pollens and plant-derived foods.
- Responsible for OAS in vegetable allergy in many cases
- There are now available two profilins: Bet v2 from birch and Phl p 12 from timothy grass in CAP system.

Calcium-binding proteins (Polcalcins or Two-EF-hand)

- Calcium-binding proteins (two EF-hand allergen or polcalcins) have been identified in pollens of numerous plants (trees, grasses, weeds).
- These allergens are remarkable because they are produced preferentially in pollen but not in other plant tissues.
- Polcalcin from various pollens - alder: Aln g 4; birch: Bet v 4; olive: Ole e 3; ash: Fra e 3; Bermuda grass: Cyn d 7; timothy grass: Phl p 7; rape: Bra n 1, 2, Bra r 1, 2- contain cross-reactive IgE epitopes.
Calcium-binding proteins (Polcalcins or Two-EF-hand)

- Must be considered as markers for broad pollen sensitization, but are not implicated in allergy to plant-derived foods.
- Marker of severity? –Ole e 3, Allergy 2002-
- Probably, as for profilin, polcalcins is not a relevant allergen as symptoms inducer?
- There are now available two polcalcin: Bet v4 from birch and Phl p7 from timothy grass in CAP system.
Parietaria Pollen-Specific Marker Allergens

- The major allergens from P. judaica pollen are Par j 1 and Par j 2 (sequence identity of 45%) both LTPs

- Clinical allergy to parieta ria in mediterranean countries has been associated with presence of sensitization to rPar j 2 (JACI 2003)

- Therefore both major allergens may be useful marker to distinguish patients genuinely sensitized against Parietaria pollen from sensitization to Parietaria whole extract in Mediterranean (Int Arch Allergy Immunol. 2005) and non-mediterranean patients allergic to other pollens and also is they are suited for specific immunotherapy with Parietaria

- rPar j 2 are available in CAP system

Olive pollen Specific Marker Allergens

- Ole e1 is one of the major allergens in olive allergic patients and a good maker of specific sensitization

- May cross-react with other major allergens from plants belonging to the Oleaceae (ash, lilac, privet) or Eleagnus angustifolia (Russian olive).

- Usefulness of other allergens (Ole e 7, 9..)?- IT reaction?

- nOle e1 will be available in CAP
Recombinants Allergens and Olive: spanish experience.

- Pacientes con alergia a olivo (Jaén): (ELISA) a Ole e 1 en el 84%; a Ole e 2 en 61.3%; a Ole e 3 en 31.9%; a Ole e 6 en 39.4%; y a Ole e 7 en 41.2% (Allergy 2001).
- Asociación entre alergenos de olivo y alergia a alimentos:
  1) SPT positivo a Rosaceae y Ole e 3 (P = 0.045) y Ole e 7 (P = 0.03);
  2) Cucurbitaceae y Ole e 7 (P = 0.03)
  3) Actinidiaceae con Ole e 3 (P = 0.04)
  4) La sensibilización a profilina de olivo (Ole e 2) no es más frecuente en pacientes con OAS
  5) pacientes con reacciones anafilácticas con frutas están sensibilizados a Ole e 7 (LTP) y tienen polinosis clínica
  6) la polalcina (Ole e 3) se asocia a síntomas respiratorios y alergia a alimentos. (Allergy 2002).

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www.alergomurcia.com
**Acacia pollen: (“an allergy mirage”)**  
*(Ann Allergy A&I, 2006)*

- 64/149 Patients (AR) with + skin test
- 5/10 +nasal challenge test
- The prevalence of sensitization to rChe a 2 -profilin-, rChe a 3 –polcalcin-, and rNtD of Ole e 9 -1,3-beta-glucanase- was 60%, 33%, and 87%, respectively, among patients sensitized to R. pseudoacacia pollen.
- Binding of IgE to R. pseudoacacia extract was completely inhibited by Robinia, Chenopodium, Olea, Cupressus, and Lolium extracts.
- The high prevalence of R. pseudoacacia pollen sensitization in patients with pollinosis is likely to be due to cross-sensitization to panallergens (profilin, polcalcin, and 1,3-beta-glucanase) from other common pollens. This phenomenon may lead to a diagnosis of "allergy mirages."

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**Panallergens Specific Marker Allergens**

**LTPs (lipid transfer proteins)**

- Nonspecific lipid transfer proteins (nsLTPs) have been described as predominant Rosaceae fruit, and some nut allergens (hazelnut, chestnut), cereals ? in the Mediterranean population.
- A high? proportion of patients sensitized to LTPs develop systemic symptoms , but on other occasions oral allergy syndrome (OAS).
- In contrast, pollen-related food allergens as PR-10 or Bet v 1 homologues from hazelnut, apple and celery and profilin in fruits most typically cause only OAS.
- LTPs are probably capable of sensitizing by the ingestion route.
- The high stability to proteolytic digestion of nsLTPs is a possible reason for more severe and systemic reactions compared with other allergens that are digestible in gastrointestinal fluid.
Panallergens Specific Marker Allergens 
LTPs (lipid transfer proteins)

- Some pollens have LTPs major allergens, *Platanus h*, *Parientaria j.*, *Olive (Ole e 7)*

- Cross-reactivity among different LTPs (eg. Cor a 8, the LTP from hazelnut, Pru av 3 from cherry, Cabbage lipid transfer protein Bra o 3, nCit s 3 from orange) is relatively high.

- IgE-mediated allergy to LTPs is a potentially severe condition, mainly in patients from the Mediterranean area, that can present with plant food and pollen allergies.

- Pru p 3 from peach will be available in CAP system, as well as rPar j 2 from Parietaria pollen.

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**Allergic reaction to rosacea fruits**

Clinical history (+)

Specific IgE (+) by SPT and/or CAP

profilin (+)

rPru p·3 (-)

Pollen allergy

Only OAS

No systemic symp

Elimination diet

Seldom to need rescue medication

No adrenalin

**Pollen allergy**

Likely OAS

No pollen allergy common systemic reactions (2/3)

With/without OAS

Pollen allergy

OAS (2/3)

Systemic sympt (1/3)

Strict elimination diet

Always recommend rescue medication

Adrenalinautoniyect

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CCD (Cross-reactive Carbohydrate Determinants)

- In most allergen sources, and especially from the plant kingdom, glycan-related IgE-reactivities have been demonstrated.

- All plant products must be suspected to contain IgE-reactive glycoepitopes: pollens, foods, latex, etc.

- Since glycoepitopes can share significant structural homologies beyond the limits of protein families they are prone to extensive cross-reactivity and they have, consequently, been called Cross-reactive Carbohydrate Determinants or CCDs.

- The level and the frequency of CCD crossreactivity is directly dependant on the degree of homology between the glycan chains present in the cross-reacting extracts.

- The main origin of IgE antibodies to CCDs is sensitization to pollens, especially in multiple pollen sensitized patients.

CCD (Cross-reactive Carbohydrate Determinants)

- Because pollen and food sensitization are often associated, it is difficult to distinguish the proper part of food glycoepitopes in the observed IgE-reactivity.

- So far the opinion has prevailed that these epitopes have no clinical impact. However, in certain subgroups of patients CCD reactivity may have clinical relevance.

- A simple way to determine a possible IgE-reactivity to CCD in an *in vitro* IgE assay is to perform an ImmunoCAP™ CCD MUXF3 test. The MUXF3 determinant is a purified glycopeptide from bromelin.
CCD (Cross-reactive Carbohydrate Determinants)

- A CCD; MUXF3 test could be useful when in vitro results do not match the clinical picture (symptoms, skin tests), especially in three types of situations:

- Sensitization to foods of plant origin, mainly vegetables and fruits, but also seeds such as peanuts

- Sensitization to *Hevea* latex in a pollen allergic patient without occupational risk factors

- In subjects tested positive for *Hymenoptera* venoms, or in subjects allergic to these venoms and tested positive for pollens.

CRD IN LATEX ALLERGY

- The pattern of sensitization to different latex allergens may vary depending of how patients are sensitized.
- IgE response to Heb v1 and Heb v3 (both have cross-reactivity) is common in patients with multiple surgeries or patients with spina bifida.
- IgE response to Heb v5 and Heb v 6.01 is seen in patients with multiple surgeries or in sensitized health care workers.
- Latex profilin (Heb v8) is considered of negligible importance in natural rubber latex extracts and as a significant allergen in inducing clinical symptoms.
- The presence of sensitization to latex profilin (Hev b8) likely means that the patient has been sensitized by other sources (plant foods and pollens).
- Prediction of systemic reactions to latex immunotherapy- levels of Hev b 6 (Allergy 2006)

- There are now available rHev b 1, 2, 3, 5, 6.01, 6.02, 8, 9, 11 in CAP system
CRD IN PET ALLERGY

- Fed d1 and Can f1 and 2 are the more relevant allergens in patients sensitized to cat and dog and are markers of clinical sensitization.

- Albumins from other animals are cross-reactive allergens and responsible for positive skin tests to several animals danders but without clinical relevance.

- **Fel d1, Can f1 and 2, and cat, dog, horse albumin is available in CAP system**

CRD IN ASPERGILLUS fumigatus ALLERGY

- IgE sensitization to *Aspergillus fumigatus* has been associated to patients that suffer from asthma or allergic bronchopulmonary aspergillosis (ABPA).

- The combination of rAsp f 1, rAsp f 3 and rAsp f 5, is 97% sensitive for diagnosing allergic asthma in patients sensitised with *A. fumigatus*.

- The inclusion of the minor recombinant allergens, rAsp f 7 and rAsp f 10, do not significantly contribute to the improvement of the sensitivity in the diagnosis of these patients.

- On contrary, rAsp f 2 rAsp f 4 and rAsp f 6 allergens are recognised by specific IgE antibodies of sera of patients with ABPA and not by sera of allergic patients sensitised by *Aspergillus fumigatus*.

- There are now available recombinants from Asp f 1, 2, 3, 4, 6; in CAP system
Tropomyosin

- The only known major allergen in crustaceans (shellfish, such as shrimp, lobster, crayfish and crab) is the muscle protein tropomyosin.

- It has been demonstrated IgE antibody cross-reactivity among tropomyosins from different crustacean species and other edible invertebrates or nematodes which is (in some cases) consistent with observed clinical sensitivity patterns.

Tropomyosin

- It has a much less prominent role in sensitization to house dust mite or cockroach, whose allergenicity is dominated by other components (10% in mites allergic patients).

- However, sensitization to tropomyosin has been implicated in associations between allergy to house dust mite and seafood, in particular following allergen immunotherapy with mite extract.

- It role in *Anisakis* s. sensitization: very small (Spain)

- rPen a 1 –tropomyosin- from shrimp (*Peneaus aztecus*) is available in CAP system
SOY AND PEANUT ALLERGY

- Recent publications described patients with birch pollen-associated allergy to legumes.

- The responsible allergens were identified as Gly m 4 in soybean and Ara h 8 in peanut (Bet v1 homologues).

- On these subjects its legume allergy might be acquired after a primary sensitization to birch pollen allergens on the basis of IgE cross-reactivity.
Sensitization to soy cannot be demonstrated and symptoms & clinical observations may be caused by factor(s) other than soy. Further investigation needed.

Test for possible cross-reactivity: e.g. ImmunoCAP™ f13 Peanut, ImmunoCAP™ Ro214 CCD/MUX3 from Bromelin or ImmunoCAP™ rPhl p 12; profilin (recombinant).

Suggested IgE ab testing of suspected soy allergic patients

Blood testing with ImmunoCAP™ f14 Soya bean

- Uncertain clinical history
- Test negative
- Convincing clinical history
- Test positive
- Uncertain clinical history

Test with ImmunoCAP™ Rf353 rGly m 4

- Test negative
- Test positive

Conclusion

- Sensitization to soy cannot be demonstrated and symptoms & clinical observations may be caused by factor(s) other than soy. Further investigation needed.
- Soy related symptoms probably due to IgG antibodies to Gly m 4. In birch pollen sensitized patients the IgG antibodies may originate from sensitization to Bet v 1.
- Sensitization to soy is present and probably underlies symptoms & clinical observations.
- Positive soy test result might be due to cross-reactivity of antibodies and the clinical relevance has to be further investigated.

Sthaph enterotoxin A, B, C, D, TSST

- Atopic dermatitis
- Cystic fibrosis
- Sinusitis
- BNCO
- Asthma
- Rinitis