Challenges for Allergy Diagnosis in Regions with Complex Pollen Exposures

Domingo Barber · Araceli Díaz-Perales · Mayte Villalba · Tomas Chivato

Abstract Over the past few decades, significant scientific progress has influenced clinical allergy practice. The biological standardization of extracts was followed by the massive identification and characterization of new allergens and their progressive use as diagnostic tools including allergen microarrays that facilitate the simultaneous testing of more than 100 allergen components. Specific diagnosis is the basis of allergy practice and is always aiming to select the best therapeutic or avoidance intervention. As a consequence, redundant or irrelevant information might be adding unnecessary cost and complexity to daily clinical practice. A rational use of the different diagnostic alternatives would allow a significant improvement in the diagnosis and treatment of allergic patients, especially for those residing in complex pollen exposure areas.

Keywords Allergy diagnosis · Pan-allergen · Component-resolved diagnosis · Pollen allergy · Food allergy

Introduction

In contrast to tropical areas, where mites are the leading and almost sole cause of respiratory allergy, in temperate regions, such as Europe or North America, pollen plays a major role in allergic patient sensitization.

There are important differences in pollen sensitization profiles. Grasses, mainly from the Poaceae family, are the most frequent pollen sensitizers. In north and central Europe, birch pollinosis ranks second in allergy triggering and together with grasses account for most of the pollen-related allergic symptoms [1].

Mediterranean and dry areas present quite different pollen exposure patterns. In addition to grasses, other pollens, such as Oleaceae, Amaranthaceae, Cupressaceae, Asteraceae, and Urticaceae, play a significant clinical role [2, 3]. In North America, there are pollen regions coincident with European ones with the added complexity of greater tree diversity and a high incidence of ragweed pollinosis [4, 5]. Ragweed pollinosis was exported at the beginning of the twentieth century to central Europe and has steadily increased since then [6, 7]. Other pollinosis caused by plants originating in desert regions are also of increasing importance in semi desertic areas.

Pollen extracts used for diagnosis consist of the complex mixtures of major allergens (sensitizing more than 50 % of the patients allergic to a particular pollen), minor allergens, with a lower prevalence, pan-allergens, and non-allergic molecules [8•]. Major allergens are relatively abundant molecules that are good markers of primary sensitization in general. Minor allergens may be of importance for identifying differential, often more severe, clinical phenotypes and rarely sensitize patients independently from major allergens [9].

Pan-allergens are highly conserved molecules present in practically all pollens (and, in some cases, in vegetable foods) and are highly cross-reactive [10–12]. As a consequence,
patients sensitized to pan-allergens will yield positive extract-based diagnosis to multiple pollens and foods.

Biological standardization consist of assigning a potency to an extract that is related to the biological activity in a dose-response skin test procedure performed on a representative sample of allergic patients [13–16]. While frequent sensitizers, such as grasses or birch, can be easily standardized, pollens with low prevalence in the allergic population are difficult to standardize and can be biased, at least partly, to pan-allergen-sensitized populations [2]. It can be estimated that at least 20% of pollen-allergic patients are sensitized to pan-allergens and will yield positive skin responses to almost any pollen extract. These extracts will be highly unspecific, and they add further noise to an already-complex diagnostic situation.

The combination of complex pollen exposure to many pollens, pan-allergen prevalence, and poorly standardized extracts often makes the correct diagnosis of allergic patients resident in these areas by extract-based diagnosis almost impossible [17]. Thus, there is a misperception of extreme polysensitivity and an urgent need to simplify and improve diagnosis in daily clinical practice.

The increasing availability of new in vitro diagnostics incorporating single-allergenic molecules either as single-allergen components, multiplexed, or in microarrays has impacted clinical practice in allergy diagnosis allowing a more accurate identification of leading sensitizers [18, 19••, 20–25]. However, there are still some problems that must be overcome. The high price of most of the available methods makes it desirable to define a rational use, as many health care providers cannot finance their indiscriminate use. Incomplete, redundant, or inadequate molecular panels, non-automated procedures, non-validated allergens, and poor-quality molecules make mass application of these protocols difficult and reduce their efficacy since an expert scientific background is required to make a reliable diagnosis. We will review available information in order to identify pragmatic diagnosis approaches using a combination of existing procedures and technologies to improve current practices for specific allergy diagnosis with a focus on patients living in complex pollen regions.

### Skin Prick Testing Diagnosis

Skin prick testing constitutes the primary allergy diagnostic tool, providing quick and relevant results, being also relatively inexpensive. Introducing single-allergen components in skin prick test, daily clinical practice would constitute a significant improvement. However, regulatory burdens make a massive molecule-based skin prick test (SPT) commercialization almost impossible, at least in the near future. However, it would be feasible to introduce a limited number of new approaches that might allow the identification of pan-allergen reactors [2, 26, 27]. By doing this, the subset of patients that cannot be diagnosed by SPT might be quickly identified, allowing a better diagnosis in the remaining patients. Profilin and lipid-transfer protein (LTP) SPT diagnostics are already available in some European countries. Profilin is the most frequent pan-allergen sensitizer, and it is normally linked to grass pollinosis [3, 28••] with a prevalence increasing along grass pollen gradients, that is, with increased grass pollen counts that includes both pollen peaks and pollinization period and is associated with a characteristic food allergic phenotype that might also be a good indicator of grass pollen allergy severity. In some areas, profilin prevalence reaches 60% of pollen-allergic patients. In regions with a lower profilin prevalence but with multiple pollen allergies, testing with profilin might allow the identification of 10–15% of patients that cannot be diagnosed by SPT and would consequently enable a quick diagnosis of most patients by extract-based skin prick test.

If pan-allergen diagnostics are not available, a good alternative would be to use an extract made from a pollen that is irrelevant for the resident population, such as a palm tree pollen extract [27]. Positive response to such an extract would mark unspecific responses to multiple pollens, so alternative diagnostic procedures might be advisable. Negative palm tree pollen response indicates a reliable SPT procedure to the remaining pollens. Unspecific SPT responses might be due to pan-allergens, residual pollen cross-contamination, or even to cross-reactive carbohydrate determinant (CCD) reactivity in some cases.

Apart from controls for unspecific reactions, the selected panel of pollen extracts should be designed according to the pollens in the area avoiding the use of redundant cross-reactive extracts [29].

### Major Allergen Markers and Cross-Reactivity

Major allergen sIgE-based diagnosis is an extremely valuable diagnostic tool. First, major allergens sensitize most allergic patients to a particular pollen; second, and more importantly, diagnoses and specific therapies are standardized and adjusted in relation to the major allergens [30–35]. As a consequence in most cases, major allergen-based diagnosis will be enough to correctly diagnose and, thus, adequately select extracts to treat more than 90% of allergic patients. Patients that are not sensitized to major allergens or pan-allergens do exist but normally represent less than 10% of the allergic population, and there is little evidence of the effect of major allergen-adjusted therapies in this type of patients.

The following is a brief summary of both major and clinically relevant minor pollen allergens and their cross-reactivity that can be used for the diagnosis of patients resident in complex pollen areas.
Grasses

Grass allergy to the Pooidae subfamily can basically be diagnosed with two major allergens Phl p 1 and Phl p 5 [36-40]. All major allergens belonging to this subfamily present a very high similarity degree [41, 42], and there is scientific evidence that a single species or even single major allergen isomorph can be successfully used for patient treatment [43-46].

About 10% of grass allergen patients can be negative to Phl p 1 and 5 and sensitized to other grass allergens such as Phl p 2, Phl p 4, or Phl p 11.

Prevalence of Phl p 1 is very high (close to 90%), while Phl p 5 prevalence, depending on the areas, ranks from 50 to 80%. Pan-allergen profilin behaves as a minor grass allergen, and its prevalence is associated with Phl p 5 [3]. Recently, severe profilin-mediated food allergy reactions have been described [28*]. Interestingly, slgG4/slgE levels to Phl 5 and Phl p 2 were lower in patients suffering severe reactions. Profilin sensitization and food allergy symptoms induced by profilin correlates with grass allergy severity [28*]. It is important to mention that profilin slgE readouts should be made globally without taking into account slgE values to the different profilins to assign profilin sensitization procedence. In some diagnostic systems, such as ISAC-CAP, several profilins are included with very different performance. For instance, rPhl p 12 normally shows a much lower sensitivity, probably in relation to inadequate isoform election or incorrect folding.

Other allergenic grasses belonging to Panicoideae (Johnson grass and Bahia grass) or Chloridoideae (Bermuda grass) lack group 5 allergens but do have group 1 allergen that displays a high (around 70%) sequence identity with Phl p 1. In extensive epidemiological studies testing both Cyn d 1 and Phl p 1, very few patients positive to Cyn d 1 and negative to Phl p 1 were found [47]. To date, there is no clinical evidence for the incremental effect of adding any of the non-Pooideae subfamily species in immunotherapy formulas in grass-allergic patients. Biological standardization procedures performed on these latter species will overdose group 1 allergens compared to Pooideae, where group 5 allergen greatly contributes to biological potency. Basically, for species lacking group 5, the extracts will be tested in the same grass-allergic population, but the concentration of group 1 allergens will be increased to compensate for group 5 absence as the target of biological standardization is to induce a defined quantitative biological response (weal area). Consequently, up to 40% of grass-allergic patients that are group 1 monosensitized might react stronger to Bermuda, Bahia, or Johnson grass extracts, without any clinically relevant implication. In commercial platforms, rPhl p 1 is included together with nCyn d 1; thus, strong CCD reactors might yield positive reactivity to nCyn d 1 and negative to rPhl p 1, the former being unspecific. Moreover, we will be comparing a single isoform with the multiple isoforms included in a natural allergen preparation. To date, there is no major allergen marker commercially available for Johnson and Bahia grass (Sor h 1 and Pas n 1).

Polcalcins, a group of pan-allergens that includes Phl p 7, sensitize between 5 and 10% of pollen-allergic patients and are not associated with any particular pollen. It is a diagnostic confounding factor and, when found as a sensitizer together with profilin, is linked to more complex sensitization profiles and many years of disease evolution [2]. As a consequence, it should be incorporated into diagnostic panels for CRD.

Birch/Oak Pollen Allergy

Bet v 1 is the best marker of PR10 related tree pollen allergy. In the US, where there is an important contribution of oak tree pollen to tree pollen-allergic phenotypes, it might be interesting to test the homologous allergen Que a 1 as well, which is not currently commercially available in component-resolved diagnostic (CRD) platforms.

Several PR10 molecules are incorporated in some arrays used for CRD, both pollen and food related. Therefore, a global interpretation should be performed evaluating food allergy in the context of PR10 allergy. Practically all patients with strong Bet v 1 recognition will yield a positive response to most PR10 allergens due to cross-reactivity to Bet v 1 (or Que a 1).

Oleaceae

Major olive pollen allergen Ole e 1 can be used as a marker of sensitization. Ole e 1 is fully cross-reactive to ash tree major allergen Fra e 1, so olive extract can be used to diagnose and treat ash tree pollen-related allergy, which is relatively prevalent in some areas of central Europe [48, 49]. The same similarity degree is also shown by the other six sequences recently analyzed in ash pollen corresponding to the counterpart in olive pollen [50]. The broad family of Ole e 1-like proteins contains members in many if not all types of pollens, but neither their relations nor their allergenic relevance are as close as those between Ole e 1 and Fra e 1.

Patients living in areas overexposed to olive tree pollen become sensitized to minor allergens, displaying a different and more severe phenotype. The inclusion of minor allergens such as Ole e 7 (belonging to non-specific lipid-transfer protein (nsLTP) family) and Ole e 9, olive pollen β-glucanase, is necessary to identify these patients [51]. In fact, in these extremely exposed areas, there are a significant proportion of patients negative to Ole e 1 and positive to Ole e 7. These could even present negative skin prick test responses to olive pollen because of the low concentration of this minor allergen in the whole pollen extracts [52].
Cupressaceae

Cup a 1, Cup s 1, and Jun a 1 are major allergens of different cypress species with a very extensive cross-reactivity [53]. As a consequence, any of them can be used to identify cypress sensitization. In Japan, where Japanese cedar allergy is very prevalent [54], Cry j 1 should also be added. To date, there is no efficient procedure to clone and express cypress allergens, and as a consequence, natural forms are incorporated in CRD procedures with the corresponding lack of specificity due to CCD cross-reactivity [55, 56]. In the interpretation of these results, other glycosylated molecules such as njug r 2, nPh p 4, or the MUXF3 CCD marker should be taken into account.

Amaranthaceae (Salsola and Chenopodium)

The relevance of these allergies, typical from desert regions, is increasing in countries as Spain because of their resistance to saline soils and dryness. Sal k 1 is a marker of Salsola allergy (Russian thistle) [57] that is the leading sensitizer of this pollen family. The inclusion of an allergen such as Che a 1 [58], cross-reactive between the different Salsola and Chenopodium species, contributes to the diagnosis of Amaranthaceae allergic patients.

Asteraceae (Artemisia, Ambrosia)

Asteracean pollen, particularly ragweed, is a main cause of clinical allergy over extensive North American regions. Art v 1 for Artemisia species and Amb a 1 for Ambrosia (ragweed) allow a correct identification of most of Asteraceae-sensitized patients [59, 60]. Art v 3 is a lipid-transfer protein of Artemisia pollen partly cross-reactive with Pru p 3, the main LTP syndrome marker. Sensitization to Art v 3 in the absence of Art v 1 should be interpreted in the context of LTP allergy.

Urticaceae (Parietaria)

Par j 2 is the main marker of Parietaria sensitization [61], highly prevalent in Mediterranean countries. In spite of being an nsLTP, as also occurs with the olive pollen nsLTP, Ole e 7, Par j 2 is not cross-reactive to any other known allergen [62].

Plantaginaceae (Plantago)

Plantain allergy is difficult to assess as the pollen season overlaps with that of grasses. There is a specific marker, Pla 11 [63], an Ole e 1-like member, that is available for specific diagnosis. Epidemiological studies suggest that plantain allergy is relevant in some areas [2, 3].

Platanaceae (Platanus)

Pla a 1 and Pla a 2 [64, 65] identify Platanus sensitization. In areas with very high exposure to this pollen, as Barcelona [66], Pla a 3, the LTP from the pollen [67], sensitizes some patients. As this protein has partial cross-reactivity with food LTPs, its sensitization should be evaluated in the context of LTP allergy as to Art v 3.

Non-specific Lipid-Transfer Proteins (nsLTPs)

LTP-mediated allergy is a complex food-pollen syndrome with a particularly high incidence in Mediterranean countries. Given the currently active research on LTPs, a better understanding of the real worldwide incidence of LTP allergy will be available in the coming years. As an example, Artemisia-related LTP sensitization has recently been described in northern China [68], suggesting that LTP allergy is relevant in other regions apart from the Mediterranean border. Peach LTP, Pru p 3, [69] is, in most cases, the leading LTP allergen. While some patients are only reactive to a limited number of LTPs (mainly from Rosaceae), some others develop sensitization and side reactions to multiple LTPs, in what is known as the LTP syndrome [70, 71]. Exposure to cross-reactive pollen containing LTPs seems to correlate with a higher recognition LTP pattern. Currently, a panel of different LTPs from foods and pollens is available for diagnosis, but the interpretation of these results is often complex.

Sensitization and Clinical Relevance

SPT-based epidemiological surveys together with aerobiological pollen data [1, 7, 68, 72–75] in correlation with allergic symptoms have been the basis for defining clinically relevant sensitizations. Apart from the previously described problems associated with extract-based diagnosis, it is not always possible to define the relevant allergens for a particular population and even more difficult to make a choice of the clinically relevant sensitizers for a particular patient. Coincident pollen seasons, long and discontinuous pollination, pollens with low aerovagant capacity but able to sensitize by close contact or in areas with sustained strong winds and different sensitization threshold for different populations can at least partly explain the complexity of the problem.

CRD offers a new tool to understand the complex dynamics underlying patient sensitization. In a systemic epidemiological study performed throughout Spanish territory, more than 2000 patients homogenously distributed over the territory were sampled from the allergic population [2, 3]. The results obtained allowed the main sensitizers to be identified in the
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G grass, O olive, C cypress, P Parietaria, S Salsola, A Artemisia, Pl Plantago, Pla Platanus, B birch, G + O + 1, 2 combinations of three or four sensitizations including grass and olive. An alternative way of analyzing the data consists in classifying patients according to the frequency of different regions, according to prevalence to major and minor allergens. By clustering patients using such a way and considering only the free three sensitization frequencies, we can highlight the distribution of the most frequent sensitizations in each patient. The results are then presented in Table 1.
In dry areas with extensive orchard tree cultivation (Teruel, Huesca) especially peach trees, a significant fraction of patients with seasonal respiratory symptoms are monosensitized to Pru p 3, the major allergen of peach and a severe food allergen. This finding demonstrates a new entry gate to fruit LTP-mediated allergy and supports the link between food and respiratory allergies. In fact, the three most common allergies affecting adults are related to PR10, LTPs, and profilin, all of them connected to respiratory allergies.

Knowing in advance the dynamics of sensitization will help to define local diagnosis algorithms which will allow an improved and effective diagnosis of allergic patients [2].
CRD is an increasingly appreciated tool that should complement traditional diagnostic procedures to improve daily clinical practice. Besides available methods, all of them complex, time consuming, and relatively expensive, there is a need for quick, inexpensive in vitro diagnostic tests adapted to the different patient sensitization profiles. For example, a test for Phl p 1.5, Ole 1, Ole e 7, profilin, and polcalcin would offer a high diagnostic resolution capacity in most of the Spanish territory. In a similar way, Phl p 1, 5, Bet v 1, profilin, and polcalcin would simplify pollen diagnosis in north and central Europe. This last panel with the inclusion of Amb a 1 would significantly improve pollen allergy diagnosis in North America. In this context, recent product diagnosis development such as multiplexed assay recently commercialized [77] might be of help.

Conclusions

Available data support that patients are usually sensitized to not more than three or four primary sensitizers and that, normally, it is possible to select one or two clinically relevant ones for specific treatment. Correct diagnostic approaches to identify clinically relevant sensitizers are possible with the existing tools available today.

Sensitization analysis alone will never be enough as it is necessary to carry this out in a clinically relevant context. For this purpose, aerobiological data interpretation, knowledge of patient sensitization patterns, and a deep understanding of the connection of allergens with associated allergic diseases is mandatory and constitutes the basis of allergy specialty.

Compliance with Ethics Guidelines

Conflict of Interest  Domingo Barber, Araceli Diaz-Perales, Mayte Villalba, and Tomas Chivato declare that they have nothing to disclose.

Human and Animal Rights and Informed Consent  This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance


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