

A Molecular Diagnostic Algorithm to Guide Pollen Immunotherapy in Southern Europe: Towards Component-Resolved Management of Allergic Diseases

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Abstract

Correct identification of the culprit allergen is an essential part of diagnosis and treatment in immunoglobulin E (IgE)-mediated allergic diseases. In recent years, molecular biology has made important advances facilitating such identification and overcoming some of the drawbacks of natural allergen extracts, which consist of mixtures of various proteins that may be allergenic or not, specific for the allergen source or widely distributed (panallergens). New technologies offer the opportunity for a more accurate component-resolved diagnosis, of benefit especially to polysensitized allergic patients. The basic elements of molecular diagnostics with potential relevance to immunotherapy prescription are reviewed here, with a focus on Southern European sensitization patterns to pollen allergens. We propose a basic algorithm regarding component-resolved diagnostic work-up for pollen allergen-specific immunotherapy candidates in Southern Europe; this and similar algorithms can form the basis of improved patient management, conceptually a 'Component-Resolved Allergy Management'.

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Introduction

Immunoglobulin E (IgE)-mediated allergic respiratory diseases, namely allergic rhinitis/rhinoconjunctivitis and asthma, represent a major health issue in most developed countries [1, 2]. From a mechanistic point of view, the central role of inhalant allergens in both disease pathogenesis and the elicitation of symptoms are indisputable. However, allergen avoidance is generally thought to be impractical and/or ineffective, both in the prevention of allergy and the induction of clinical remission in established disease, as most trials have failed to show significant benefit from avoidance measures [3, 4].

Given the limited effectiveness of such a directly causal approach, pharmacotherapy, mainly the use of antihistamines, bronchodilators and anti-inflammatory agents, is considered the cornerstone of treatment [1, 5]. Nevertheless, allergen-specific immunotherapy (SIT) represents an important intervention, currently adjunctive to pharmacological treatment, which confers specific advantages over conventional management. SIT has been shown to exhibit disease-modifying potential, with a clinical benefit that outlasts treatment duration

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[6, 7], and has been attributed preventive capacity against the progression of allergic rhinitis to asthma and the development of IgE-sensitization to additional allergens [8, 9].

Although much remains to be elucidated regarding underlying mechanisms in different types of SIT, it is a common understanding that SIT acts in an allergen-specific manner [10]. It should therefore be anticipated that the efficacy of SIT may be highly dependent on both the ability to correctly identify the culprit allergen(s) and on the availability and concentration of the relevant allergens in therapeutic extracts. This represents a considerable challenge as diagnostic extracts, whether intended for serological (in vitro) or skin-prick (in vivo) testing, are traditionally based on crude extracts of the allergen source, thus containing a mixture of allergens, relevant and not relevant. The same applies for immunotherapy vaccines, as recombinant allergens are currently not licensed for therapeutic use in humans, and all commercial immunotherapy products are extracts of the raw allergenic materials, containing variable levels of both allergenic and nonallergenic substances. Moreover, information is scarce regarding the relative concentration of specific allergens in natural extracts, either diagnostic or therapeutic, while methodological heterogeneity is the rule for the sporadically published reports [11–13] on the topic; this further complicates the issue of matching immunotherapy prescription to specific IgE-sensitization patterns and might at least partially account for some observed ineffectiveness in immunotherapy trials and in routine practice.

Recently, a major step forward in the direction of diagnostic optimization was signaled by the introduction of component-resolved diagnostics (CRD) [14]. Analyzing patient serum IgE-specificity to the level of single allergens is now possible, enhancing our overview of the IgE-sensitization profile; this would be expected to facilitate selection of candidates for immunotherapy and administration of the appropriate extracts. However, to our knowledge, since the introduction of component-resolved diagnosis, only a few published articles exist on how to use the corresponding results for the optimal selection of immunotherapy candidates [15–18]. This is of prime importance in the Mediterranean region; in contrast to the more or less clearly separated pollination periods of major allergenic plants in the north of Europe, the overlapping flowering periods of allergenic plants in the south, in conjunction with an abundance of multisensitization profiles, frequently hamper diagnosis and make it difficult to assess the clinical relevance of a given IgE

sensitization [15, 16]. It is possible that such a diagnostic work-up may also be applicable in regions across the world with similar aerobiological characteristics, for example, in parts of Australia or in the southern USA (e.g. Florida).

Although the published reports provide a valuable first insight in the utilization of these exciting new techniques, they mainly focus on distinguishing patients sensitized to species-specific allergens from those sensitized to cross-reactive allergens. The basic principle is that identification of IgE-sensitization to a species-specific allergen is a marker of genuine sensitization, rules out positivity due to cross-reacting components and is therefore a prerequisite for the selection of a therapeutic extract of the corresponding natural source. It is possible, nonetheless, that other elements may also be incorporated in such an analysis, like available published data on the relative allergen representation in commercial extracts, confounding factors in in vitro diagnosis and potential markers indicating a reduced anticipated response to specific immunotherapy. By taking the above points into account, we attempt to build upon existing algorithms and propose a practical framework for optimized immunotherapy candidate selection, with a focus on the most important allergenic pollen sources in the Mediterranean. This and other similar algorithms can form the basis of improved patient management, conceptually a 'Component-Resolved Allergy Management' (CRM).

Pollen Allergy

There is general consensus that SIT should be indicated in cases of established clinical relevance of an allergen source [19]. When seasonal symptoms point to pollen allergy, in vivo and/or in vitro testing typically confirm the presence of IgE to the specific pollen. In cases of IgE-sensitization to more than one pollen source, it is important to identify the clinically significant pollens and exclude any source that may appear positive exclusively by cross-reactivity, as misrecognizing the primary sensitizing source(s) may in turn compromise immunological responses to SIT. This is especially relevant in southern Europe, where a clinical history may be suboptimally informative, owing to the partial overlap of the pollinating periods of grasses, trees and weeds.

A number of assays are now routinely available, detecting IgE towards pollen allergen components. These

can be classified to those signifying genuine sensitization to a given source (species-specific allergens) and others, indicating broad cross-sensitization.

Markers of Genuine Sensitization to Major Pollen Sources

Grasses

Grasses are the only allergenic pollen species with ubiquitous representation and clinical significance across Europe. Therefore, it is not surprising that half of all commercially marketed CRD assays for inhalant sources involve grass allergens. Among these, Phl p 5 and Phl p 6 do not exhibit significant serological cross-reactivity to pollen allergens outside the Pooideae subfamily, and are thus regarded as species-specific allergens for Timothy grass (*Phleum pratense*) and all other Pooideae members, including common meadow grass (*Poa pratensis*), orchard grass (*Dactylis glomerata*) and perennial ryegrass (*Lolium perenne*) [20]. IgE to Phl p 2 may also be regarded as fairly specific for patients sensitized to grass species of the Pooideae subfamily; although Phl p 2 can also be found in non-Pooideae grass species (e.g. *Zea mays* and *Cynodon dactylon*), its immunological importance seems to be confined to the Pooideae subfamily [21].

When IgE specific to one or more of these component allergens is detected and the clinical history suggests grass allergy, grass pollen immunotherapy is indicated. Identification of the exact Pooideae member inducing primary sensitization may not be as important; both mixed grass pollen extracts and single representative species (*P. pratense* pollen) have been argued to be effective [22–25].

Phl p 1 represents the archetype of group 1 grass pollen allergens and is the most prevalent allergen among grass-allergic patients [26, 27]. However, the corresponding IgE is only partially specific for the Pooideae grass subfamily, dominating in Europe; partial cross-reactivity between Phl p 1 and Cyn d 1, the group 1 major allergen in Bermuda grass (*C. dactylon*), frequently hampers correct identification of the sensitizing allergen source [28]. Specifically, when testing for Pooideae-specific molecules (e.g. rPhl p 2, rPhl p 5 and rPhl p 6) is negative, relatively higher levels of IgE specific to nCyn d 1 than to rPhl p 1 have been suggested to be indicative of primary sensitization to Bermuda grass [29]; a SIT extract containing Bermuda grass pollen might then be suitable, especially as such an extract may be quantitatively standardized according to group 1 allergen (nCyn d 1) content. In con-

trast, if antibodies against Pooideae-specific molecules (e.g. rPhl p 5) are positive and specific IgE levels against rPhl p 1 are higher than those to nCyn d 1, the case is most probably primary sensitization to Pooideae grasses and Bermuda pollen representation can be omitted from the SIT regimen. Finally, if both conditions are present (i.e. group 5 antibodies-positive and specific IgE against nCyn d 1 higher than to rPhl p 1), the clinician may be facing a true double sensitization.

It should not be overlooked that clinical response to immunotherapy relies on adequate representation of sensitizing allergens in SIT extracts; in this regard, the concept of maximum tolerated extract dose is advocated [30]. Most manufacturers determine the concentration of only one major allergen as part of the standardization process [12]; information regarding additional allergen content is generally not available. It is expected, therefore, that if IgE-sensitization proves positive against the same major allergen that is employed in extract standardization, this would ensure the enhanced possibility of response to SIT. This is underscored by the fact that the levels of major allergen content and the biological potency of extracts have repeatedly been found to correlate well [13, 31]. In this context, detection of anti-rPhl p 5 IgE, compared to other grass-specific markers, has added value in terms of associated grass-immunotherapy outcome, as rPhl p 5 quantification is the rule for such standardization by companies and Phl p 5 is typically found in abundance in extracts [12, 13, 28, 31, 32].

Trees

In a similar fashion to grass pollen CRD, markers for better response to immunotherapy can be utilized for the most important allergenic tree pollen in southern Europe. nOle e 1/rOle e 1 and nCup a 1 are specific markers for olive and cypress pollen, respectively, and are available for routine testing. Potential positivity suggests authentic sensitization and validates selection of the corresponding natural SIT extract, when compatible symptoms are present. Briefly, Ole e 1 is the most frequent sensitizing allergen in olive pollen [33]; it is also typically used in the standardization process of olive pollen diagnostic and therapeutic extracts, therefore it is not surprising that Ole e 1 is the only allergen yet proven to induce immunological changes during olive pollen SIT [34]. Ole e 1-like molecules are also present in grass (Phl p 11), chenopod (Che a 1), English plantain (Pla l 1) and saltwort pollen (Sal k 5) [35]. It seems, however, that cross-reactivity of Ole e 1 with the molecules outside the Oleaceae family is limited, probably nonsignificant [36, 37]. Importantly, and owing

to the high cross-reactivity between Ole e 1-like molecules within the Oleaceae family, positivity to rOle e 1 may serve as a marker of the suitability of ash or privet pollen SIT in olive-free areas with a high exposure to pollen of these closely related Oleaceae members [36–38].

nCup a 1 is the only available diagnostic marker for primary sensitization to pollen of the Cupressaceae family. Due to the structural homology of their major allergens, genuine sensitization to either *Cupressus arizonica* and/or *C. sempervirens*, both prevalent in the Mediterranean basin, may be confirmed by this single assay [39]. In this case, the high level of cross-antigenicity between members of the Cupressaceae family may justify therapeutic administration of a single representative pollen extract, even if it is not the same as the originally sensitizing species [40].

Plane tree (*Platanus acerifolia*) pollen can be a cause of respiratory allergy in some patients. Major allergens rPla a 1 and nPla a 2 are commercially available only through the ISAC allergen biochip immunoassay (Thermo Fisher Scientific, Uppsala, Sweden) which, unfortunately, is not very widely used. Positive specific IgE (sIgE) against these components may serve as a marker of primary sensitization to plane tree pollen [41, 42]. In contrast, rPla a 3 is a minor allergen that belongs to the nonspecific lipid transfer protein (LTP) family and has been linked with IgE-sensitization to plant-food LTPs [43].

Weeds

Wall pellitory (*Parietaria judaica* and *P. officinalis*), mugwort (*Artemisia vulgaris*) and, lately, saltwort (*S. kali*) are regarded as the most prevalent allergenic weeds in the Mediterranean [44–46]. With respect to wall pellitory allergy, Par j 1 and Par j 2 have been described as major allergens in *P. judaica* pollen [47, 48]. Although they both belong to the largely cross-reactive LTP allergen group, they in fact lack significant cross-reactivity to other pollen or plant-food LTPs, thus conferring specificity of induced IgE for the *Parietaria* genus [49–51]. However, only rPar j 2 is commercially available for routine testing. Theoretically, this would have an impact on the assay's sensitivity, i.e. the assay's ability to detect weed-allergic patients genuinely sensitized to *Parietaria* pollen. Nonetheless, detection of IgE to rPar j 2 alone has been proven to be sufficient in more than 80% of cases [48], probably owing to Par j 1 and Par j 2 sharing at least three identical allergenic epitopes [47]. The reported structural similarity is primarily held to account for the demonstrated potency of Par j 1-standardized *Parietaria* pollen extracts in anti-rPar j 2-IgE-positive patients [52]. By taking

the above into account, clinicians may use rPar j 2 as an efficient *Parietaria*-specific marker when considering weed-specific immunotherapy.

Although both nArt v 1 and nArt v 3 have been reported to be species-specific molecules in mugwort-allergic patients and potential markers of positive response to SIT [29], the role of sensitization to the nsLTP Art v 3 may not be as straightforward. There are conflicting data regarding the role of Art v 3 as a primary sensitizer. The selection of the population is a probable reason for this, but what seems to be of prime importance is the geographic area under study; in European (Mediterranean) populations, Art v 3 may be of little clinical relevance, generally considered to reflect primary sensitization to cross-reacting plant-food LTPs [53–56]. In contrast, cross-reactivity between plant-food and mugwort LTP has been shown to be inversely directed in selected patients and in patients in northern China [57, 58]. In any case, the total content of Art v 3 in crude pollen material has been shown to strikingly vary from batch to batch, hardly being detected in some [51]; this would put into question the reliability/sufficiency of SIT natural extracts for patients sensitized only to Art v 3. For the above reasons, the role of detecting specific IgE to nArt v 3 in selecting immunotherapy candidates remains rather controversial at the moment.

On the other hand, nArt v 1 is considered a true major mugwort pollen allergen, accounting for IgE-sensitization in approximately 80% of mugwort-pollen-allergic patients [59, 60]. Importantly, it is an effective marker of genuine sensitization to mugwort pollen, even when cross-reactivity to minor ragweed allergen Amb v 4 occurs [61]. Furthermore, when major allergen quantification is involved in the process of mugwort pollen extract standardization, Art v 1 is typically used, suggesting a potential increase in SIT efficacy in anti-Art v 1-IgE-positive patients [12].

Finally, commercially marketed nSal k 1-IgE assay may provide a species-specific marker for primary saltwort pollen allergy, which is steadily gaining significance across extensive areas in southern Europe due to ongoing desertification [46, 62]. Quite importantly, detection of specific IgE to nSal k 1 is able to distinguish the majority of patients sensitized to saltwort from those sensitized to closely related chenopod (*Chenopodium album*) pollen [63]. As both weeds pollinate at overlapping periods and often appear double-positive in extract-based diagnostics, identification of the primary sensitizing source may be of added value, allowing more specific immunotherapy [63].

Markers of Cross-Reactivity: Panallergens

In essentially birch-free southern Europe, profilin and polcalcin molecules are the main cause of in vitro and in vivo cross-reactivity across a broad spectrum of allergenic pollen. The highly conserved structure and ubiquitous distribution of these molecules among plant species are the basis for their ascribed 'pan-allergenicity' [64]. Unfortunately, this presents additional challenges, when it comes to evaluating the sensitization profiles of immunotherapy candidates; profilin- and polcalcin-sensitized patients tend to recognize these molecules in numerous sources and, as a consequence, score positive in extract-based diagnostic testing with most pollen species [65–67]. Analysis by CRD partially resolves this problem. In fact, only a limited number of pollen panallergens are available for routine use (grass profilin, rPhl p 12, and birch profilin, rBet v 2; grass polcalcin, rPhl p 7 and birch polcalcin, rBet v 4), but due to marked structural homology among allergenic species, these serve as efficient markers of hypersensitivity to the entire group of homologous proteins, with the possible exception of *Parietaria* and cypress profilins [68–72]. The fact that grass pollen is the most prevalent cause of both profilin and polcalcin sensitization in the Mediterranean, along with an excellent cross-reactivity spectrum of both molecules, favors the use of the grass-derived allergens over the birch-derived ones, at least in this geographic region [66, 68, 70, 73].

The presence of IgE-sensitization to such panallergens has been suggested to be a marker of poor outcome in pollen immunotherapy, especially in the absence of IgE to species-specific molecules [16, 20]. In our opinion, sensitization to pollen profilins and/or polcalcins would be expected to decrease, at least to some extent, the efficacy of SIT for the following reasons.

i) Whether profilin and polcalcin are clinically relevant allergens is an ongoing matter of debate; recently, it was demonstrated that they may actually elicit symptoms in sensitized patients [74–76]. Moreover, the content in various pollen SIT extracts, at least for profilin, has been shown to be remarkably low [74], meaning that profilin-sensitized patients are expected to receive a therapeutic extract of little immune-modulatory capacity against profilin.

ii) Sensitization to both profilin and/or polcalcin allergens has been associated with a longer duration of allergic disease and with resulting cosensitization to a larger number of species-specific allergen molecules, compared to patients that do not recognize panallergens [46, 65, 66]. This increases the number of genuine sensitiza-

tions to different pollen and would theoretically require multiple pollen representation for immunotherapy to be effective.

iii) Sensitization to both profilin and/or polcalcin allergens typically follows previous cosensitization to other molecular allergens from the same pollen source, as has been elegantly shown in the grass pollen model [77, 78]. It is proposed that, at least for grass pollen allergy, panallergens are only being recognized at the late stage of this 'molecular spreading', where the pattern of molecular sensitization is more complex and the favorable window of 'early immunological intervention' has probably passed [77, 79]. This is especially relevant, since therapeutic extracts do not contain all relevant allergens at high doses [32]; at best, the concentration of just one major allergen is ensured [12].

In summary, there are theoretical grounds to suggest that the detection of anti-rPhl p 12-IgE and/or anti-rPhl p 7-IgE may reduce the anticipated response to pollen SIT, due to associated molecular multisensitization and the practical inability to administer an 'appropriate', allergen-matching therapeutic extract. However, in case sIgE against major allergens is present, SIT with extracts containing these allergens can be administered, especially as the clinical relevance of profilins and polcalcins is still arguable.

The Role of Cross-Reactive Carbohydrate Determinants

Cross-reactive carbohydrate determinants (CCDs) are not only present in the glycoproteins of virtually all pollen, but are regarded as the most frequently encountered individual epitope structures for IgE [80]. Nonetheless, after years of debate about their actual participation in the induction of symptoms, there is now enough evidence to support the view that IgE-sensitization to CCDs is clinically insignificant, as these carbohydrates seem to be able to activate basophils by IgE-binding only ex vivo [80, 81]. Either due to insufficient representation, low binding-affinity of IgE to carbohydrate epitopes or the presence of CCD-blocking IgG4 antibodies, glycans do not behave as allergens in vivo [80, 81]. In contrast, IgE to CCDs does become evident by 'contaminating' extract-based in vitro specific-IgE testing; widespread positivity is then the rule [82].

If these apparently multisensitized patients undergo CRD, there is a similar possibility of misleading, false-positive results when glycosylated component allergens

are utilized. Such CCD-containing natural purified glycoproteins include nCyn d 1, nOle e 1, nCup a 1, nSal k 1, nPla a 2 and nArt v 1, used for the detection of primary sensitization to Bermuda grass, olive, cypress, saltwort, plane tree and mugwort pollen, respectively. On the contrary, recombinant forms of proteins are typically produced in the reducing cytoplasmic environment of *Escherichia coli* bacteria, which is unable to host posttranslational modification, and thus the glycosylation of proteins [83]. As a result, IgE to CCDs does not interfere with recombinant CRD pollen allergens. It must be noted, nonetheless, that the lack of posttranslational modifications in *E. coli*-produced recombinant allergens may theoretically result in improper folding of the allergen protein, leading to potentially false-negative results due to reduced allergen recognition by the patient IgE [83].

When considering CRD results in the formulation of SIT extracts for apparently multisensitized patients, it is imperative to exclude potentially CCD-restricted IgE-sensitization to natural glycosylated species-specific allergens, i.e. the presence of IgE directed *only* to the carbohydrate moiety in the absence of IgE specific for the protein fragment. Available markers of sensitization to CCDs are bromelain (nAna c 2) and MUXF3, the purified N-glycan from bromelain (on a peptide backbone) [29]. These are able to detect IgE to N-glycans in most pollen sources. When the clinician is confronted with positive in vitro results to a natural glycoprotein, negative sIgE results to CCD markers reveal the protein nature of IgE epitopes. An exception is the case of positivity to nArt v 1; IgE-sensitization to CCD may not be excluded by testing with bromelain or MUXF3, as these markers detect IgE to N-glycans, whereas nArt v 1 is known to primarily contain O-glycans [84]. On the contrary, positive sIgE results to CCD markers should optimally be accompanied by assessment of biological activity, i.e. by positive skin-prick testing or nasal/conjunctival challenge with the allergen source [80, 81]. This approach may serve to prove the concomitant presence of sIgE against protein epitopes, and thus validate the inclusion of the allergen source in the SIT regimen.

A Clinical Algorithm for Using CRD in Pollen-Specific Immunotherapy: Introducing 'Component-Resolved Management'

The use of a panel of species-specific allergen markers, representing the most common allergenic species in the Mediterranean, along with the panallergen screening

molecules rPhl p 7 and rPhl p 12, may facilitate the selection of those immunotherapy candidates who would have an increased probability of benefitting from SIT. A proposed clinical algorithm for the assessment of pollen-allergic patients suitable for extract-based immunotherapy in the birch-pollen-free areas of southern Europe is depicted in figure 1.

Initially, a basic diagnostic discrimination between mono/oligo- and multisensitization may be performed, based on skin test readings and/or sIgE in vitro results using common pollen extracts. In the case of mono- or oligosensitization, an individual assessment of patient suitability for SIT is carried out on the basis of sensitization to major species-specific molecules that are contained in commercial therapeutical extracts in as close to 'high dose' as possible.

In a multisensitized pollen-allergic patient, an additional assessment of (co)sensitization to panallergens is important, as detection of sIgE directed towards profilins and/or polcalcins would possibly mean a poorer outcome of immunotherapy for the reasons discussed above. With respect to species-specific markers, when nonglycosylated recombinant allergens are used in in vitro tests, positive sIgE results to major grass-specific rPhl p 5/rPhl p 1, *Parietaria*-specific rPar j 2 or olive-specific rOle e 1 may justify immunotherapy with the respective pollen extract. In the case of positive in vitro results for Bermuda grass, cypress, olive, saltwort or mugwort pollen major allergens (nCyn d 1, nCup a 1, nOle e 1, nSal k 1, and nArt v 1 respectively) in multisensitized patients, sensitization restricted to the CCD component must be optimally ruled out, as these molecules are used in diagnostics as purified natural CCD-rich isoforms. As the role of CCDs in clinical allergy is generally disputed, it would be of questionable value to specifically target these molecules; only in the case of concurrent sensitization to a protein component would immunotherapy to the allergen source be a prudent choice.

Conclusion

We propose a CRM plan for pollinosis in southern Europe, based on molecular sensitization patterns for the most common pollens in the area. At the moment, there are no prospective studies to evaluate the added benefit of applying CRD in terms of SIT efficacy. However, in a prospective study, it has recently been shown that the incorporation of CRD results was able to alter initial SIT indication and allergen prescription in more than half of the

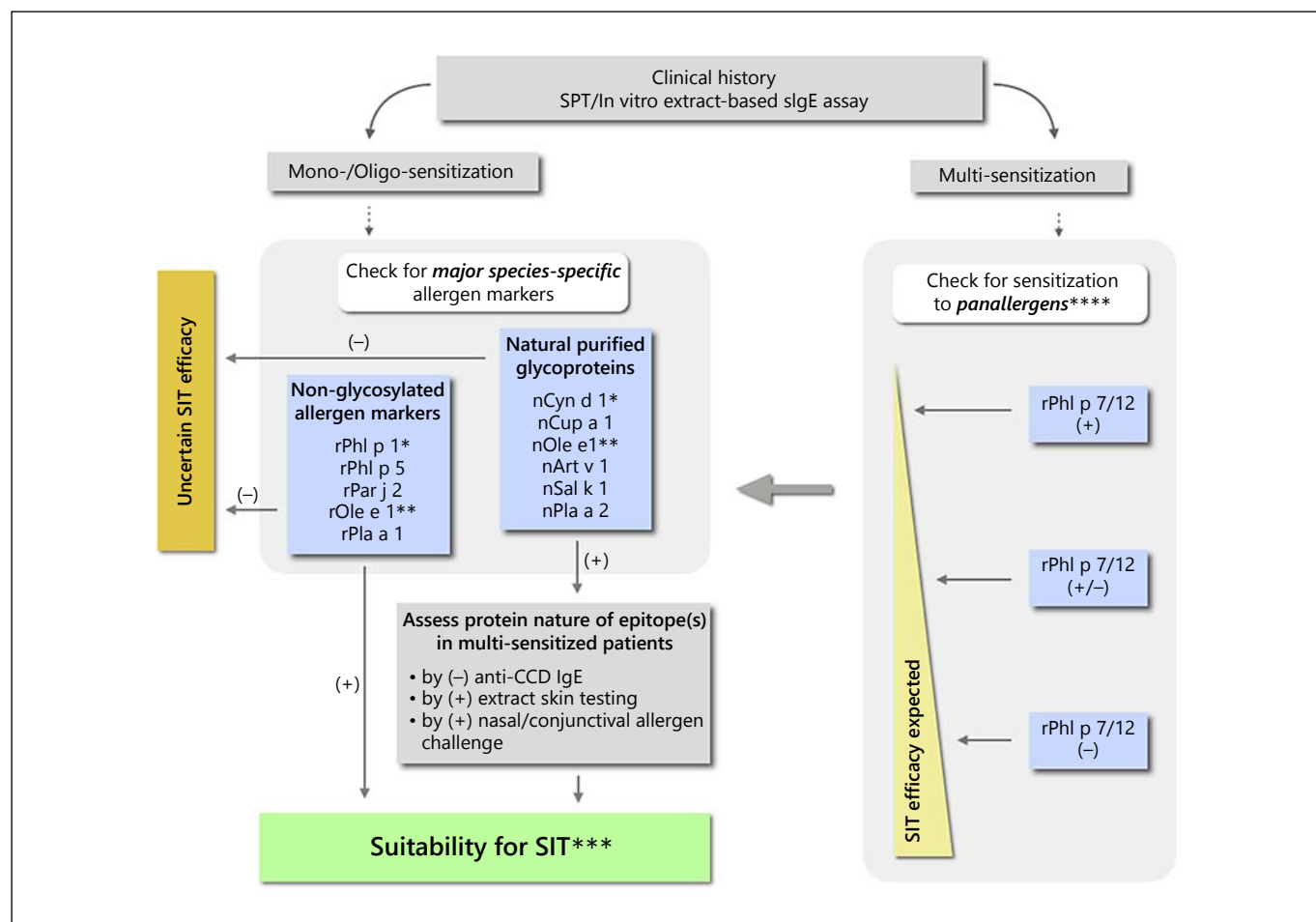


Fig. 1. Proposal for a basic CRD work-up for pollen SIT candidates in southern Europe. For the purposes of the current figure, component allergens' names denote the detection of corresponding specific IgE. * In cases with nCyn d 1 (+) > rPhl p 1 (+) and negative species-specific allergen markers for Timothy grass (e.g. rPhl p 2, rPhl p 5 and rPhl p 6), a Bermuda grass standardized extract may be sufficient for the treatment of clinical allergy to grass pollen (please refer to text). ** Both natural nOle e 1 and recombinant

rOle e 1 are commercially available in Europe. *** For optimal SIT efficacy, the use of standardized extracts containing the species-specific major allergen at high-dose concentration may be required. **** rPhl p 7, as a polcalcin marker and rPhl p 12, as a profilin marker are typically used. In areas with a high prevalence of birch pollen allergy (not typical of the Mediterranean region), rBet v 4 and rBet v 2 may respectively be used, along with the essential rBet v 1 birch pollen major allergen.

patients studied [85]. Moreover, results from a retrospective analysis of SIT efficacy with respect to molecular sensitization patterns favor such an approach and are compatible with the underlying rationale [18].

Of course, for the moment, these remain mainly theoretical considerations; significant limitations include incomplete and sometimes conflicting published data, inadequate information about the allergen content of commercial SIT extracts as well as reported qualitative and quantitative variability between different SIT products and manufacturers [12, 31, 86–88]. In addition, taking

into account the limited number of allergen components currently available for clinical use and the fact that exact matching of natural SIT extracts to individual sensitization profiles may probably be a Utopian goal, one could reasonably argue against the practical value of such an attempt. However, until patient-tailored recombinant extracts are licensed for clinical use and until prospective controlled studies provide solid data on all significant aspects, we argue that CRM, based on the best-available scientific knowledge, may enhance our diagnostic and therapeutic accuracy.

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