

Multiple Pollen Sensitization: A Molecular Approach to the Diagnosis

Adriano Mari

Allergy Unit, National Health Service, Rome, Italy

Key Words

Asthma · IgE · Pollens · Recombinant allergens · Skin tests

Abstract

Background: Sensitization to multiple pollen species is a frequent diagnostic event. Several allergenic molecules with a high level of homology have been identified in divergent pollen families and named panallergens. **Methods:** We sought to define the criteria to evaluate the prevalence of the multiple pollen sensitization, to identify specific markers of this condition, and to correlate them with the underlying allergic disease. Patients presenting an allergic respiratory disease underwent skin testing with 23 pollens. Patients fulfilling predefined selection criteria were grouped and classified as having multiple pollen sensitization. Patients in each subgroup were tested for IgE to rBet v 2, rJun o 2, rBet v 1, rPhl p 5 and bromelain. Demographical, allergological and clinical data were recorded in the subgroup of patients with multiple pollen sensitization. **Results:** Seventeen percent of the pollen-sensitized patients formed the multiple pollen-sensitized subgroup. These subjects were positive for most of the pollen species tested regardless of known exposure to them. None of the subjects sensitized to less than six pollen species were positive to panallergens, whereas 55% of the sera of the multiple pollen-sensitized group were positive to rBet v 2, and 15% to rJun o 2. IgE to rBet v 1 and rPhl p 5 were found positive in all the subgroups. Age, gender, bronchial asthma, oral allergy syndrome, skin test reactivity and previous specific im-

munotherapy differed significantly when these two subsets were considered. **Conclusions:** Allergy diagnosis based on allergenic molecules is crucial in the patient with multiple pollen sensitization. This condition appears to be determined by the sensitization to defined allergenic components (panallergens) rather than by pollen of multiple species as such. Detection of IgE to nonpanallergenic molecules allows to identify more relevant allergenic sources. Clinical aspects of the underlying allergic disease (e.g. asthma and oral allergy syndrome) seem to be differently related to IgE reactivity to panallergens.

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Introduction

Hypersensitivity to pollens is one of the most common aspects of the allergic respiratory disease, leading to rhinitis, conjunctivitis and asthma [1]. Subjects with pollinosis can be sensitized to a single or to several pollen species depending on their exposure to worldwide or to regional pollens [2, 3]. Sensitization to multiple allergens, pollens among them, has been reported to lead to more serious symptoms and to increase the risk of asthma [4–6]. In a previous study [7] we recorded a high prevalence of skin prick test (SPT)-positive results to several pollen species belonging to taxonomically distant families. The analysis of the diagnostic data showed that more than 60% of the subjects were sensitized to pollen of two or more species and 15–20% reacted to most, if not all, the pollen species tested (unpubl. data). At the moment, criteria to discriminate these groups of patients are not yet defined, although

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Correspondence to: Dr. Adriano Mari
Via Malipiero 28
I-04100 Latina (Italy)
Tel. +39 0330 860005, Fax +39 0773 620787
E-Mail a.mari@panservice.it

positivity to panallergens has been suggested as a possible specific marker of multiple pollen sensitization (MPS) [8–10].

In the last decades, an increasing number of purified natural or recombinant allergens have been fully characterized in terms of physicochemical characteristics, biochemical properties, sequence homology and taxonomy [11, 12]. Several studies focused the possible replacement of the allergenic extracts with the recombinant allergenic molecules for diagnostic purposes [13–16]. Besides protein allergens, cross-reacting carbohydrate determinants (CCDs) are recognized by IgE antibodies and have been proposed to cause the widest pattern of in vitro cross-reactivity even among molecules with unrelated protein sequence [17, 18]. The poor functional activity seems to be an important property of these determinants [19], thus influencing the clinical value of IgE detection [18].

The aim of this study was to evaluate the overall degree of the allergic sensitization to pollens of a population suffering from allergic respiratory disease. Their reactivity to several pollen species differing in relation to exposure level was studied in detail. We tentatively defined criteria to identify the patients with MPS, and evaluated the IgE reactivity of the subjects against allergenic molecules with different taxonomic distribution and associated allergic diseases. The working hypothesis was that each of the selected molecules (e.g. panallergens for MPS patients) could be the marker of a reactivity pattern to pollen extracts. The molecules chosen for the study were rPhl p 5, rBet v 1, rBet v 2, rJun o 2 and CCDs. Phl p 5 is an allergenic protein from *Phleum pratense* (timothy grass) with a high level of homology with proteins (denominated group 5 allergens) present only in the grass family [11, 14]. Bet v 1, the major allergen of birch, has a high level of homology with other proteins from species of the Betulaceae, Corylaceae and Fagaceae families, all belonging to the Fagales order [20]. Some foods contain allergenic molecules with a high level of homology to Bet v 1, like Mal d 1 in apples, Api g 1 in celery, Dau c 1 in carrot [21]. This can contribute to the oral allergy syndrome and other food allergy-related disorders elicited when foods containing Bet v 1-homologous molecules are eaten by patients primarily sensitized to Bet v 1 from birch pollen [22]. Profilin, an actin-binding protein firstly identified as an allergen in birch (Bet v 2) [23], has homologous counterparts in a high number of pollens from phylogenetically distant botanical families, which has led to define profilins as panallergens [8]. The presence of a profilin-like molecule in several foods is the cause of the food allergy related to pollen sensitization [22]. Jun o 2, a calcium-binding aller-

genic protein, has recently been identified, characterized and cloned [10] and, together with other allergens with the same biological activity [9], is likely to belong to a second group of panallergens. In fact, calcium-binding proteins are essential factors in all higher biological systems [9], and several functionally homologous molecules have already been identified from other pollen sources [9, 10]. At the moment, no data are available about the presence of this group of molecules in plant-derived foods. The presence of CCDs-IgE was studied to better define their relevance among the pollen-allergic population and their possible role in the MPS [18]. Finally, demographical, allergological and clinical data, collected from patients with MPS, were analyzed to tentatively associate clinical differences among subgroups with an IgE reactivity at the molecular level.

Materials and Methods

Allergenic Extracts, Purified and Recombinant Allergens

The pollen species selected for the study (table 2) have been regarded as representative of the corresponding botanical family, and reflected the widest possible range of known allergenicity and exposure levels. The selection criteria were the following. (1) Species which are present in the Mediterranean area (rye grass, juniper/cypress, olive, pellitory) [2, 7]. (2) Species which are present in the urban and suburban areas where the patients live and work as suggested by aerobiological pollen sampling [2] and environmental surveys (elm, goosefoot, hazel, lime, maple, mugwort, oak, pine, plantain, poplar, sycamore) [24, 25]. Some of these species have been reported to cause pollinosis in geographical areas other than central Italy (hazel, goosefoot, maple, mugwort, plantain) [2, 3], or to sensitize subjects with elevated but restricted environmental exposure (elm, lime, sycamore) [3, 26]. (3) Species which are rare or not present at all in the studied area (birch, mustard, ragweed, salt cedar, tree of heaven) [24, 25], or have been seldom, if ever, reported as sensitizers (eucalyptus, locust tree, salt cedar, tree of heaven) [3], or produce a very small amount of pollen (amphiphilous or entomophilous plants) for a short period of time (2–4 weeks/year) (eucalyptus, locust tree, salt cedar, tree of heaven), or are rare ornamental plants (palm, salt cedar, tree of heaven) [24, 25]. Although cross-reactive components have been described in ragweed and mugwort extracts [27], both belonging to the Compositae family, they have been considered separately on account of their distribution in different geographical areas [2, 3], and their divergent prevalence recorded in our preliminary study (unpubl. data). Although it is not present in Italy, mountain cedar (*Juniperus ashei*) pollen extract was chosen as the most cross-reactive diagnostic species among the Cupressaceae family [7]. Allergenic glycerinated extracts for SPT were obtained from Allergopharma (Reinbek, Germany; birch, elm, goosefoot, rye grass, hazel, lime, locust tree, maple, mugwort, oak, olive, pellitory, plantain, poplar, ragweed, sycamore and the not-pollen extracts excluding cockroach) and Bayer (Spokane, Wash., USA; eucalyptus, juniper/cypress, mustard, palm, pine, salt cedar, tree of heaven and cockroach). Bromelain was purchased from Sigma-Aldrich (Milan, Italy), and tested before it was used on human beings [18].

Recombinant Phl p 5, Bet v 1, Bet v 2 molecules, used for IgE detection, were available on the ImmunoCAP system (Pharmacia & Upjohn, Uppsala, Sweden). Recombinant Jun o 2 was produced by the Laboratory of Immunology of the Istituto Superiore di Sanità (Rome, Italy) according to Tinghino et al. [10].

Patients, Symptoms, Skin Testing and Sera

Unselected subjects (n = 2,503) presenting respiratory symptoms that suggested an allergic disease, and referred to the Allergy Unit in the period of 1996–1999 were enrolled during their first consultation. The patients who had previously received a specific immunotherapy (SIT) course were not excluded from the study. A SIT course was considered when at least 1 year of treatment was achieved. All the treated patients received alum-adsorbed extract.

In the subgroup of patients defined as having an MPS, all reporting rhinitis and conjunctivitis, seasonal symptoms of asthma were recorded on the basis of their own history and of a follow-up consultation during the subsequent pollen season. The grading of asthma severity was done on the basis of NHLBI/WHO criteria [28]. The presence of an oral allergy syndrome to foods was recorded following previously defined clinical criteria [29]. On the basis of the personal history of seasonal symptoms, the starting age of pollinosis was retrospectively defined.

Patients underwent skin testing, with a standardized procedure [30], for the 23 pollen species listed in table 2 and for bromelain. Bromelain was used at 500 µg protein/ml as already reported [18]. They were also tested for mites, cockroach, cat, dog, horse, *Alternaria alternata*, *Aspergillus fumigatus*, *Candida albicans*, *Cladosporium herbarum*, *Penicillium notatum* and *Tricophyton mentagrophytes*. A result was considered positive when a wheal of at least 3 mm greater than the negative control was recorded 15 min after the application of the test. Positive results were scored and recorded in four classes: class 1, when the wheal diameter was one fourth to half the histamine wheal, class 2, when it ranged from half to about the same size as in the positive control, class 3, from equal to twice as large as the histamine skin reaction, and class 4, greater than twice the positive control.

After the SPT, sera were obtained from all the patients who consented to blood sampling for an in vitro diagnostic procedure. Sera matched for age and sex distribution those of the original subgroups. They were stored at –20 °C until required. Informed consent for skin testing and blood sampling was obtained from patients during their allergy consultation.

Grouping Criteria

Patients with negative SPT to all the allergenic extracts were recorded as having a nonallergic respiratory disease (n = 754, 30.1%). Patients with positive SPT were divided in two groups: *Pollen* (positive SPT to at least one pollen species; n = 1,294, 74%) and *Not-Pollen* (n = 455, 26%). Patients belonging to the *Pollen* group were further divided into two subgroups on the basis of the following criteria: (1) positive SPT for more than 50% (twelve) of all the tested pollen extracts, (2) at least 3 positive SPT for pollens either rare or absent in the studied area (pollens from selection criteria 3 in the first section of Materials and Methods), and (3) at least 1 positive SPT for one of the relevant pollen species (pollens from selection criteria 1 in the first section of Materials and Methods). Patients matching all the criteria were considered to have MPS and are indicated as the PAN subgroup, whereas the remaining ones represent the Not-PAN subgroup. The patients in the Not-PAN subgroup were further divided

in those having a positive SPT for a single pollen species (the MONO subgroup), those with 2–5 positive SPT (the OLIGO subgroup), and those having more than 5 positive SPT but matching only one or two of the selection criteria defining the PAN subgroup. The latter represented a borderline subgroup indicated as BORDER. Grouping and subgrouping criteria were arbitrarily defined on the basis of preliminary studies, and set up to define a possible diagnostic relationship between SPT reactivity patterns and IgE reactivity to the allergenic molecules considered.

IgE Detection

Serum IgE to rBet v 1, rBet v 2, rPhl p 5 and bromelain were detected by the ImmunoCAP system following the instructions of the manufacturer (Pharmacia & Upjohn); values equal to or greater than 0.4 kU_A/l were considered positive. Discrepancy of the results between a positive in vitro test to bromelain, a glycoprotein bearing carbohydrate moieties recognized by IgE, and a negative SPT to the same glycoprotein was indicative of the presence of CCDs-IgE [18]. IgE to rJun o 2 were detected by ELISA as reported elsewhere [10]. A cutoff equal to 0.1 OD₄₉₀ value (mean of ten normal sera plus three standard deviations) was established.

Results

About 74% (n = 1,294) of the patients with an allergic respiratory disease were sensitized to at least one pollen species (*Pollen*). Table 1 reports the overall results obtained when pollen-allergic patients are grouped according to the criteria indicated in the Materials and Methods section. About 1 out of 6 subjects in this group, 17.6% (n = 228) of the total subjects allergic to pollen, met the criteria to be classified as having MPS. Mean age at the time of the allergy consultation, and range limits did not differ significantly among subgroups. Twenty-two percent of the subjects in the PAN subgroup were aged under 12 years. The male to female ratio differed significantly only between allergic and nonallergic patients (data not shown).

Table 2 reports the results of the prevalence of the 23 pollen species in the SPT for each subgroup. The prevalence of grass, cypress, pellitory and olive was above 7% in all the subgroups, with a marked increase from the MONO to the PAN subgroups. Mugwort and sycamore yielded a prevalence of 3.1 and 1.8%, respectively, in the MONO subgroup. All the remaining pollen species were rarely, if ever, capable of causing an isolated sensitization. Prevalences steadily increased for all the species tested in the BORDER subgroup and even more in the PAN subgroup. With the exception of elm, pine and ragweed, a prevalence greater than 50% was recorded for all the species in the PAN subgroup, and 14 of them (60.9% of 23 species) recorded a prevalence close to, or greater than, 80%. These results were not dependent on the exposure to the pollen source.

Table 1. Group distribution and demographical data of the pollen-allergic population based on SPT results

Group	Number	Pollen %	Not-PAN %	Age, years		M/F
				mean $\pm$ SD	range	
Pollen	1,294			27 $\pm$ 15	2–73	0.99
Not-PAN	1,066	82.4		27 $\pm$ 15	2–73	0.93
MONO	421	32.5	39.5	27 $\pm$ 16	2–68	0.88
OLIGO	584	45.2	54.8	27 $\pm$ 14	3–73	0.94
BORDER	61	4.7	5.7	27 $\pm$ 13	5–65	1.26
PAN	228	17.6		26 $\pm$ 14	4–70	1.3

Pollen = Subjects with positive SPT to at least one pollen extract; *Not-PAN* = subjects with positive SPT to pollens and not matching the three grouping criteria indicated in Materials and Methods; *MONO* = subjects with positive SPT to a single pollen species; *OLIGO* = subjects with positive SPT to 2–5 pollen species; *BORDER* = subjects with positive SPT to more than 5 pollen species and matching 1 or 2 of the criteria indicated in Materials and Methods; *PAN* = subjects with positive SPT to pollens and matching all the three criteria indicated in Materials and Methods. All the subgroups with pollen-positive SPT were considered regardless of their reactivity to not-pollen-allergenic extracts.

Table 3 reports the prevalence values for the allergenic molecules tested. The prevalences of sera with IgE to rPhl p 5 and to rBet v 1 were calculated for patients with a positive SPT to rye grass and to birch extracts, respectively. An increasing prevalence of sera with IgE to rPhl p 5 in the four subgroups was recorded. rBet v 1-positive sera were 75% in the OLIGO and 57% in the BORDER subgroups, dropping significantly to 28% in the PAN subgroup. None of the sera tested from the MONO and OLIGO subgroups had IgE to rBet v 2, whereas 13% of the subjects of the BORDER and 55% of the PAN subgroups were positive for the same molecule. A 15% prevalence of IgE to the rJun o 2 allergen was recorded only in the PAN subgroup. CCDs-IgE, detected by means of bromelain, were increasingly positive from MONO to PAN, with values ranging from 21 to 67%. Mean IgE values for each molecule did not differ significantly among subgroups. The statistical evaluation of the data is reported in the footnote to table 3.

Demographical, clinical and allergological parameters differed when subsets of patients reacting to either of the panallergens were determined (table 4, columns 1 and 2). Male subjects were more represented in the rBet v 2-positive subset (M/F 1.9), whereas an opposite ratio was recorded in the rJun o 2 subset (M/F 0.33). The mean age – both at the time of the allergy consultation and when the allergic symptoms presumably began – differed significantly between rBet v 2 and rJun o 2 subsets, the former being younger than the latter. None of the patients with IgE to rJun o 2 was of pediatric age at the time of the

allergy consultation. The large majority (87.5%) of the patients with IgE to rJun o 2 reported bronchial asthma, whereas only 51.7% of the rBet v 2-positive patients suffered from bronchial symptoms. Patients with severe asthma (grade III or IV) were 9.7% in the rBet v 2-positive subset and 42.8% in the rJun o 2-positive subset. Conversely, the oral allergy syndrome was rarely reported by the patients in the rJun o 2 subset as opposed to 40% of the patients in the rBet v 2 subset, which is interesting if we consider that the prevalence of rBet v 1-positive sera did not differ between the two subsets (data not shown). A difference was also recorded in the number of patients who previously received an SIT course. Twenty-three percent of rBet v 2 patients received at least a 1-year course of SIT with at least one pollen extract and this prevalence did not differ from patients in the Not-PAN subgroups (data not shown), whereas more than two thirds of the patients in the rJun o 2 subset had received the same treatment in the past. Although all the patients included in the PAN subgroup met the selection criteria indicated, those belonging to the rJun o 2 subset had a higher mean percent value of positive SPT and a ‘stronger’ skin reactivity. The presence of IgE to both molecules, recorded in 6.2% of the sera from the PAN subgroup, led to a clinical and allergological condition resembling an overlapping of the two (table 4, column 3). A further increase in the prevalence of a more severe seasonal asthma was recorded, reaching two thirds of the subjects suffering from bronchial symptoms. The double-negative subset of patients included about one third of the patients in the PAN sub-

Table 2. SPT results for 23 pollen species in grouped subjects

Allergenic source	Pollen					PAN (n = 228)
	total	Not-PAN				
	(n = 1,294)	total (n = 1,066)	MONO (n = 421)	OLIGO (n = 584)	BORDER (n = 61)	
Selection criteria 1						
Grass, rye						
<i>Lolium perenne</i> , Poaceae	66.9	60.3	37	74.3	80.3	97.8
Juniper/cypress						
<i>Juniperus ashei</i> , Cupressaceae	52.2	46.8	30	56.2	65.6	77.5
Olive						
<i>Olea europea</i> , Oleaceae	34.7	24.1	7	35.9	27.9	84.1
Pellitory						
<i>Parietaria judaica</i> , Urticaceae	39.9	36	19.6	45.8	50.8	58.7
Selection criteria 2						
Elm						
<i>Ulmus minor</i> , Ulmaceae	10.1	3.4	0	2.3	31.1	39.5
Goosefoot						
<i>Chenopodium album</i> , Chenopodiaceae	21.5	6.9	0	7.5	38.3	83.3
Hazel						
<i>Corylus avellana</i> , Corylaceae	24.4	11.1	0.8	15.2	37.9	82.4
Lime						
<i>Tilia europaea</i> , Tiliaceae	18.9	3.7	0	2.2	37.7	85.4
Maple						
<i>Acer</i> spp., Aceraceae	13.1	2.2	0	1	26.2	61.1
Mugwort						
<i>Artemisia vulgaris</i> , Compositae	21.1	13.4	3.1	16.4	52.5	57.4
Oak						
<i>Quercus alba</i> , Fagaceae	20.9	7.4	0	8.8	37.7	80
Pine						
<i>Pinus edulis</i> , Pinaceae	1.5	0.2	0	0.2	1.7	6.9
Plantain						
<i>Plantago lanceolata</i> , Plantaginaceae	24.3	10.1	0.7	13.9	31.1	86.8
Poplar						
<i>Populus alba</i> , Salicaceae	16.9	1.7	0	1.6	11.7	80.8
Sycamore						
<i>Platanus occidentalis</i> , Platanaceae	18.3	6.7	1.8	5.8	42.6	68.6
Selection criteria 3						
Birch						
<i>Betula verrucosa</i> , Betulaceae	20.5	7.5	0	10.8	26.2	80.7
Eucalyptus						
<i>Eucalyptus globulus</i> , Myrtaceae	14.3	2.4	0	2.3	16.7	66.5
Locust tree						
<i>Robinia pseudoacacia</i> , Leguminosae	20.4	5.0	0	3.1	52.5	88.2
Mustard						
<i>Brassica rapa</i> , Cruciferae	20.3	2.2	0	1.7	12	56.6
Palm						
<i>Phoenix canariensis</i> , Palmae	21.6	5.0	0	4.2	40	90.4
Ragweed						
<i>Ambrosia elatior</i> , Compositae	7.4	1.3	0	1.5	7	32.4
Salt cedar						
<i>Tamarix gallica</i> , Tamaricaceae	22.1	5	0	3	48.3	93.5
Tree of heaven						
<i>Ailanthus altissima</i> , Simaroubaceae	27.2	10.6	0	10	73.3	95.4

Common name, taxonomic name and botanical family for each pollen source are given. For selection criteria see the Materials and Methods section. Values of SPT are expressed as percentage of the total number of patients in each group.

Pollen = Subjects with positive SPT to at least one pollen extract; Not-PAN = subjects with positive SPT to pollens and not matching the three criteria indicated in Materials and Methods; MONO = subjects with positive SPT to a single pollen species; OLIGO = subjects with positive SPT to 2–5 pollen species; BORDER = subjects with positive SPT to more than 5 pollen species and matching 1 or 2 of the criteria indicated in Materials and Methods; PAN = subjects with positive SPT to pollens and matching all the three criteria indicated in Materials and Methods.

Table 3. Prevalence of IgE to rPhl p 5, rBet v 1, rBet v 2, rJun o 2, and CCDs in selected subgroups of patients with pollinosis

Group ¹	rPhl p 5	rBet v 1	rBet v 2	rJun o 2	CCDs
Not-PAN					
Total (371)					
Positive, %	47 ^a	71 ^e	1 ^g	0	33 ⁱ
Value ± SD	10.5 ± 15.5	5.5 ± 8	0.8 ± 0.3		1.9 ± 4.1
Range	0.4–70.9	0.4–39.3	0.4–1.2		0.4–33.2
MONO (132)					
Positive, %	35 ^{b, c}	–	0	0	21 ^{j, k, l}
Value ± SD	6.5 ± 9.4				0.8 ± 0.5
Range	0.4–31.6				0.4–2
OLIGO (208)					
Positive, %	48 ^d	75 ^f	0	0	38 ^{j, m, n}
Value ± SD	12.9 ± 18	6.1 ± 8.6			2 ± 4.1
Range	0.4–70.9	0.4–39.3			0.4–33.2
BORDER (31)					
Positive, %	63 ^b	57	13 ^h	0	58 ^{k, m}
Value ± SD	7.3 ± 10.7	2 ± 1.9	0.8 ± 0.3		3.1 ± 6.4
Range	0.5–33.8	0.4–4.7	0.4–1.2		0.4–25.8
PAN (177)					
Positive, %	76 ^{a, c, d}	28 ^{e, f}	55 ^{g, h}	15	67 ^{i, l, n}
Value ± SD	17.6 ± 17.2	10.3 ± 15.7	3.3 ± 5.3	1.1 ± 0.9	3.1 ± 5
Range	0.4–74.3	0.4–67.6	0.4–39.5	0.13–2.8	0.4–37

Percentages of sera with IgE to rPhl p 5 and rBet v 1 were calculated in subjects with positive SPT to rye grass and birch, respectively. CCDs-IgE were considered positive when IgE to bromelain were detected and negative SPT results were recorded for the same glycoprotein. The values of rPhl p 5, rBet v 1, rBet v 2 and bromelain tested by the ImmunoCAP system are expressed as kU_A/l (range of positive values from 0.4 to >100 kU_A/l). The values of rJun o 2 tested in ELISA are expressed in optical density (range of positive values from 0.1 to >2.8 OD₄₉₀). For grouping criteria see footnote to table 2. The groups with pollen-positive SPT were considered regardless of their reactivity to not-pollen-allergenic extracts.

Statistics: Fisher's exact test was used to evaluate data on contingency tables. Results (superscripts refer to compared couples of data marked with the same letter): ^{a, c, d, e, f, g, h, i, k, l, n} $p < 0.0001$; ^j $p < 0.002$; ^b $p < 0.02$; ^m $p < 0.05$. The low number of observations in the BORDER subgroup prevented us from doing some of the statistical evaluations.

¹ Values in parentheses represent sera.

group and their parameters are reported in table 4, column 4. The statistical evaluation of the data is reported in the footnote to table 4.

Discussion

The present study was carried out to address, from a molecular point of view, the diagnosis of patients with MPS, and to try to define specific markers of their allergological condition. In the first part of the study, the SPT with allergenic extracts was used to show how the introduction of a high number of pollen extracts with a low or irrelevant environmental exposure might be helpful in the

selection of MPS patients. The matching of the three defined criteria was assumed as sensitization to allergenic components that can be present with homologous counterparts in many plant species rather than recording the subjects' hypersensitivity to the pollen sources themselves. Patients reacting to a high number of pollen species, most of them with rare or never-described allergenicity, would be sensitized to allergenic components shared by several pollen sources. The recorded prevalence of the patients with MPS (PAN subgroup) was about 13% of all the allergic subjects and 17.6% of the pollen-allergic subgroup. About two thirds of the subjects in the PAN subgroup had IgE to at least one of the two panallergens, whereas none of the subjects in the MONO and the

Table 4. Demographical, clinical and diagnostic parameters of 177 (77.6%) out of 228 subjects with MPS (PAN subgroup)

Group	rBet v 2+ rJun o 2—	rBet v 2— rJun o 2+	rBet v 2+ rJun o 2+	rBet v 2— rJun o 2—
Subjects				
Number	87	16	11	63
%	49.1	9	6.2	35.6
M/F	1.9 ^a	0.33 ^a	0.57	1.03
Allergy consultation (age)				
Mean $\pm$ SD, years	25 $\pm$ 14 ^{b, c}	36.2 $\pm$ 15 ^{b, d}	36.9 $\pm$ 15 ^{c, e}	26.2 $\pm$ 13 ^{d, e}
Range, years	6–61	12–71	13–64	5–65
Symptoms (starting age)				
Mean $\pm$ SD, years	14.4 $\pm$ 10 ^f	22.9 $\pm$ 15 ^{f, g}	13.8 $\pm$ 11	14.3 $\pm$ 11 ^g
Range, years	2–43	4–53	2–34	2–53
Bronchial asthma ¹	51.7 ^h	87.5 ^{h, i}	81.8 ^j	44.4 ^{i, j}
Grade III–IV asthma ²	9.7 ^{k, l}	42.8 ^{k, m}	66.6 ^{l, n}	3.6 ^{m, n}
Oral allergy syndrome ¹	39.5 ^p	12.5 ^p	44.4	36.8
SIT ¹	23 ^{q, r}	68.7 ^{q, s}	72.7 ^{r, t}	27 ^{s, t}
SPT 'percentage' ³	73.5 $\pm$ 13 ^{u, w, x}	87.9 $\pm$ 8 ^{u, y}	84.8 $\pm$ 7 ^{w, z}	66.8 $\pm$ 13 ^{x, y, z}
SPT 'reactivity' ⁴	1.9 $\pm$ 0.4 ^{a, β}	2.8 $\pm$ 0.4 ^{a, χ}	2.6 $\pm$ 0.5 ^{β, δ}	1.8 $\pm$ 0.4 ^{χ, δ}

Statistics: Mann-Whitney test for comparison of mean values from unpaired data and Fisher's exact test to evaluate data on contingency tables were used. Results (superscripts refer to compared couples of data marked with the same letter): ^{u, y, α , χ} $p < 0.0001$; ^{l, n, z, δ} $p < 0.0005$; ^{i, q, x, β} $p < 0.002$; ^{a, b, r, s, t} $p < 0.005$; ^{c, e, h, k, m, w} $p < 0.01$; ^{d, f} $p < 0.02$; ^{g, j, p} $p < 0.05$. The low number of observations in the rBet v 2+/rJun o 2+ subset prevented us from doing some of the statistical evaluations.

¹ Values are expressed as percent of positive subjects.

² Values are expressed as percent of positive subjects among those with bronchial asthma.

³ SPT 'percentage' was calculated as percent of positive tests on 23 pollen extracts tested on each subject. Values are expressed as mean $\pm$ SD (range 51–100).

⁴ SPT 'reactivity' was calculated as the mean score of the SPT in each patient, considering both positive and negative tests. For scoring criteria see Materials and Methods. Values are expressed as mean $\pm$ SD (range 0.6–4).

OLIGO subgroups recognized them. The pollen extracts selected for the SPT have been shown, by the ELISA inhibition, to contain cross-reactive epitopes that inhibited the binding of IgE to rBet v 2 and rJun o 2 [31]. The fact that IgE reactivity to rBet v 2 and to rJun o 2 was limited to the PAN subgroup, and that the ELISA inhibition has been obtained by most, if not all, the pollen extracts [31] further supports the definition of these molecules as pollen panallergens. These molecules should be regarded as markers to be used in the diagnosis of an allergological condition (pollen panallergy) rather than for the diagnosis of allergy to a pollen source [14, 16, 20, 32].

Fourteen percent of the BORDER patients recognized rBet v 2, whereas none recognized rJun o 2. This data could suggest that at least part of these subjects could develop a complete MPS. Although they fulfilled the three criteria, one third of the PAN subjects reacted to neither rBet v 2 nor rJun o 2. In this group of patients, the multi-

ple pollen reactivity could be caused by certain epitopes that are present in the natural molecule and partially lost in the rearrangement of the recombinant allergen [16], or by differences in the molecular structure of homologous proteins from different sources [8]. A further hypothesis to explain the double-negative subset is the presence of IgE to other panallergens not tested in this study [33, 34] or as yet unknown. A partially overlapping IgE reactivity among identified molecules with the same biological activity (e.g. calcium-binding proteins) [9], but with a low sequence homology or different conformational epitopes could also be hypothesized. A complete panel of pollen panallergens will be useful in the molecular diagnostic approach to the MPS patient.

Prevalence of IgE to rBet v 2 was higher than to rJun o 2, and divergent clinical features seem to characterize the patients reacting to these two panallergens. The differences recorded in gender and age distribution suggest how

IgE reactivity to these molecules could be associated with different immunogenetic mechanisms. Differences in the prevalence of asthma and its severity, oral allergy symptoms, and skin test reactivity suggest a different interaction between these allergenic molecules and the mucosal district involved. IgE to rJun o 2 might have a predictive role in the clinical course of MPS toward a severe form of asthma, whereas food allergy and a milder form of asthma might be related to rBet v 2 sensitization. A combination of IgE to both molecules, merging the features of the two subsets, would further increase the level of attention for the clinical course of the underlying allergic respiratory disease.

The induction of a de novo IgE reactivity has been documented after SIT courses [35]. A higher percentage of patients only in the rJun o 2-positive subset who had previously received a SIT course could be related to the SIT itself, but more probably it is due to the severity of their bronchial disease. Longitudinal studies are needed to correctly evaluate the influence of previous SIT on MPS subjects.

Prevalence of CCDs-IgE has been reported to be about 30–40% among pollen-allergic patients [17, 18]. The data recorded on CCDs-IgE in this study – showing their increasing prevalence with the increase in the number of positive SPT to pollens – did not allow us to discriminate whether IgE reactivity to these epitopes could be the cause rather than the effect of the pollen panallergy condition. CCDs-IgE were found, with similar mean and range levels, also in those groups presenting one or few pollen sensitizations. Results obtained in these two groups confirmed the lack or low biological activity of the IgE that recognize CCDs, as reported in previous studies [18, 19]. At the same time, CCDs have been described in almost all the allergenic extracts tested using the IgE inhibition assay on bromelain [31]. If suitable models with other glycoproteins will confirm data on histamine release capabilities by isolated glycans [36], a possible eliciting role in MPS could also be ascribed to CCDs. The demonstration of their activity will increase the number of patients with MPS identified by a functional panallergenic structure.

The prevalence of rPhl p 5-IgE reactivity increased significantly among the four subgroups (35–76%), indicating a prominent and growing priming role of the species belonging to the grass family in the observed population. The mean prevalence of rPhl p 5-IgE-positive sera was very similar to that reported by other authors (about 80%) [14] only in the PAN subgroup. As in our population birch allergy was mainly a consequence of the sensitization to birch homologous allergens, rBet v 1-reacting sera were

not found in the MONO subgroup because patients allergic to birch always had a positive SPT to hazel and oak. The patients with rBet v 1-IgE formed a small but clearly defined subpopulation in the OLIGO and BORDER subgroups. The mean prevalence of rBet v 1-IgE-positive sera in these two subgroups was very close to that reported by other authors [16, 32]. The significant lower prevalence of rBet v 1-IgE in the PAN subgroup was clearly due to the higher number of patients with a positive SPT to birch elicited by the profilin or the calcium-binding proteins present in the birch pollen extract [8, 9]. From these data, the use of nonpanallergenic molecules, like rPhl p 5 and rBet v 1, in the diagnosis of MPS can be considered crucial. In fact, skin reactivity to a large number of pollens hinder the possibility of identifying species with a prominent clinical importance besides their panallergen content. The use of a panel of allergenic molecules from other families (e.g. Ole e 1, Par j 1, etc.) [11, 12] would complete this component-resolved diagnosis [37]. The allergenic molecule-based diagnosis would also help in the correct choice of an SIT treatment that should include all the allergenic extracts relevant for the patient, until allergenic molecules are available for the immunotherapy of allergic diseases [37].

In conclusion, skin testing with common and rare pollens is still necessary to define the pattern of allergenic source reactivity, but the definition of the IgE reactivity to panallergenic and nonpanallergenic molecules is the necessary step forward to correctly define the individual sensitization. Molecule-based diagnosis is of great importance to clearly define the individual reactivity of subjects with MPS. Already known panallergens are probably the first step toward the identification of a panel of molecules that lead to the development of an MPS in the sensitized patient. Identification of subjects with severe asthma related to an IgE reactivity to a defined group of biological molecules (e.g. calcium-binding proteins) could lead to a better definition of pathogenetic mechanisms of bronchial asthma.

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