

IgE Sensitization to *Thaumetopoea pityocampa*: Diagnostic Utility of a Setae Extract, Clinical Picture and Associated Risk Factors

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Key Words

Immunoglobulin E · Insect allergy · Pine processionary moth · Setae · *Thaumetopoea pityocampa*

Abstract

Background: Setae from *Thaumetopoea pityocampa* larvae (the pine processionary moth or PPM) can induce hypersensitivity reactions, but their clinical role in IgE-mediated responses is still subject to discussion. The aim of this study was to evaluate a setae extract for in vivo and in vitro diagnosis in nonhospitalized patients with reactions to PPM. **Methods:** Forty-eight adult patients presenting with PPM cutaneous reactions were studied by skin prick test (SPT) and specific IgE using setae and whole larval (WL) extracts. Biological standardized extracts were used for skin tests. **Results:** A total of 47.9% patients had a positive SPT for PPM (70% to both extracts, 17% only to the WL extract and 13% only to the setae extract). IgE immunoblotting detected several reactive bands in 91% of the SPT-positive cases. In multivariate analysis, male sex, immediate latency (<1 h) and duration of skin symptoms (<24 h) were independent predictors of a positive SPT. **Conclusions:** IgE sensitization to PPM

was found in 48% of the study patients, which was associated with immediate reactions and evanescent cutaneous lesions. Most of these patients reacted to both WL and setae extracts, but some reacted to only one of them. According to our data, skin and in vitro tests to PPM should be performed using both extracts.

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Introduction

Larvae of the pine processionary moth (PPM, *Thaumetopoea pityocampa*) constitute important urticating insects in the Mediterranean area. In addition, the PPM is expanding northwards because of the global warming, which is favoring their survival during winter [1, 2]. Larval development of the PPM includes five larval stages or instars, with all being gregarious. The 1st and 2nd instars (occurring during late summer to early autumn) are not urticating. From autumn to spring, PPM larvae pass from the 3rd to the 5th instars (L3–L5); these three instars carry urticating setae (hair-like material) on the dorsum for protective purposes. Conspicuous white silky tents, easy

to spot on pine trees, are built during autumn as protective shelters. At the end of winter, the larvae reach the last stage (L5), in which they show the highest amount of setae. After a variable period of time, the larvae move down the tree trunk in procession to the ground where they pupate under the surface. They remain there during spring, although they can remain in prolonged diapause in the pupal stage for several years. Finally, in early summer, adult moths emerge, mate and lay eggs. Human exposure to L5 larvae is more likely because the larvae leave trees at this stage. Some workers such as pinecone collectors have also symptoms in months corresponding to L3–L4 larvae and others (e.g. foresters) throughout the whole year, because setae may persist for a long time in the environment [2, 3].

Health effects may result from touching the larvae directly or, more frequently, by air contamination with setae [2]. PPM reactions include a variety of symptoms, notably urticaria and papular rashes on exposed cutaneous areas. Ocular lesions and rhinitis can also occur, whereas dyspnea and anaphylaxis are less frequent [2–11]. Nonallergic and allergic factors have been implicated in their pathogenic mechanism [3–10, 12–14]. However, several mechanisms may operate simultaneously, and the clinical role of IgE-mediated responses remains to be elucidated. IgE-mediated responses have been assessed using whole larval (WL) extract, but the presence of allergens in setae has recently been described by us [10].

The aim of this work was to study the clinical significance and risk factors of IgE sensitization to PPM in adult nonhospitalized patients with cutaneous reactions to this insect in an endemic area, and to evaluate a skin prick test (SPT) extract obtained from setae for in vivo and in vitro diagnosis.

Patients and Methods

Patients

Forty-eight adults with PPM cutaneous reactions were included in this study. They were randomly selected from a cross-sectional study including patients stratified by geographic region, age and sex; the study was performed in an area endemic for PPM in the northwest of Spain [2].

PPM cutaneous reactions were defined as (1) hives, papular rash or dermatitis within 24 h of visiting pine areas; (2) characteristic distribution of the rash on the body; (3) according to the insect's life cycle, the rash should appear at least during the months in which the caterpillar is urticating (October–April), unless it was associated with the collection of wood, pine cones or sand from a PPM-infested pine forest; in this case, dead larvae, cocoons, nests and debris from an infested pine forest can cause reactions throughout the whole year, and (4) no other cause of skin lesions.

This study was approved by the Ethics Committee of the Río Hortega University Hospital (Valladolid, Spain), and written informed consent was obtained from all patients included in the study.

Setae and Caterpillar Collection

Colonies of *T. pityocampa* were collected in Valladolid. Setae collection was performed according to a method described previously [10].

Skin Testing and Evaluation of Atopy

SPT were performed with a metal lancet using WL and setae PPM extracts (both from caterpillars in L5), according to a standardized procedure [13] and specific recommendations [14].

Biological standardization of SPT for both WL and setae extracts was previously performed. The patients were included based on the criteria for the standardization of allergen extracts of the Nordic guidelines [13]. All of them (21 patients for WL and 25 for setae extract) suffered from immediate allergic symptoms caused by PPM, and positivity was detected by IgE immunoblotting. Twenty nonexposed subjects (10 atopic and 10 nonatopic) were used as controls. PPM in the last larval stage (L5) were collected from pine trees (*Pinus pinea*) in Valladolid and immediately frozen at -20°C . Four extract concentrations of WL and setae were prepared (10, 5, 1 and 0.1 mg/ml; Bial-Aristegui Laboratory, Bilbao, Spain). SPT were carried out in duplicate with the different extract concentrations. Histamine dihydrochloride (10 mg/ml) and sterile 0.9% saline were used as positive and negative controls, respectively. The response was considered to be positive if the wheal diameter was at least 3 mm (mean wheal area 7 mm^2) higher than the negative control 15 min after puncture. The SPT value was defined as the extract concentration (in mg/ml) eliciting a wheal reaction equal to that produced by 10 mg/ml histamine hydrochloride in the same population. The wheal area was measured by digitized planimetry. SPT with 10 mg/ml of WL or setae extract was positive in all of the patients and negative in all 20 control subjects. Moreover, all of the patients had a positive SPT with 5 mg/ml of WL extract, and 5/21 and 13/21 patients had a negative SPT with 1 and 0.1 mg/ml of extract, respectively. One, 8 and 8 of the 25 patients had a negative SPT with 5, 1 and 0.1 mg/ml of setae extract, respectively. From the dose-response curve, the WL extract showed an SPT value of 2.53 mg/ml and the setae extract of 2.72 mg/ml (equivalent to 10,000 biological units/ml). After this biological standardization, we considered that the 5 mg/ml concentration was adequate for diagnostic purposes for both WL and setae extracts (equivalent to 19,763 and 18,382 biological units/ml, respectively).

The patients were also tested to a predefined panel of aeroallergens (grass, weed and tree pollen, house dust mites, molds, animal dander and cockroaches), foods (milk, hen's egg, cereals, legumes, nuts, wheat, fish, mustard, seafood and peach), *Anisakis simplex* and latex (Bial-Aristegui Laboratory).

Atopy was defined as a positive SPT reaction to at least one of the aeroallergens tested together with the presence of typical symptoms such as asthma, rhinoconjunctivitis or eczema/dermatitis [15].

SDS-PAGE and Immunoblotting

Electrophoresis was carried out on 16% Tris-tricine minigels according to standard procedures. IgE was detected by immunoblotting using a previously described diffusion method [16]. West-

ern blotting was performed using the Immuno-Star AP chemiluminescence kit and the ChemiDoc MP system (Bio-Rad Laboratories, Hercules, Calif., USA) according to the manufacturer's instructions.

Statistics

All analyses were performed using SPSS statistical software (version 12.0). Descriptive analysis of the different variables was performed as well as comparisons of means and ratios. Univariate logistic regression was performed to evaluate risk factors associated with a positive SPT. A stepwise multivariate logistic analysis (likelihood ratio) was conducted to evaluate independent predictors for a positive SPT. Probability values of $p < 0.05$ were considered statistically significant.

Results

Skin Prick Testing

SPT with PPM extracts were positive in 23/48 patients (47.9%); 16/23 (69.6%) SPT-positive patients reacted to both extracts and 7/23 (30.4%) only to one extract, 4 (17.4%) to WL and 3 (13%) to setae. There were no significant differences between the two extracts regarding wheal areas in SPT ($p = 0.234$).

Three of 23 SPT-positive patients had a positive SPT to common aeroallergens concomitant with atopic disease, and another patient had subclinical sensitization to aeroallergens. Significant differences were not found in SPT-negative patients (4/25 had atopic predisposition and none had a subclinical sensitization to aeroallergens; table 1).

Clinical Symptoms and Risk Factors

Bivariate analysis showed significant associations between patients with a positive SPT to PPM and male sex ($p < 0.05$), frequency of exposure to pine areas ($p < 0.05$) and occupational exposure ($p < 0.05$; 61% of SPT-positive subjects: 7 pinecone collectors ($p < 0.05$), 3 woodcutters, 2 gardeners, 2 bricklayers, 2 foresters, 1 sand and gravel quarry worker and 1 sports instructor). The most frequent skin lesion was urticaria ($p < 0.0001$), followed by dermatitis in non-IgE-mediated reactions ($p < 0.005$), and the most frequently affected cutaneous area was the neck ($p < 0.005$). Patients with positive SPT showed a significantly higher proportion of immediate reactions after exposure to pine areas (mean 23.69 min) than those with negative SPT (mean 201.13 min; $p < 0.0001$). Moreover, in the SPT-positive group, cutaneous lesions usually resolved by 24 h (mean 18.82 h), i.e. significantly earlier than those in the SPT-negative group ($p < 0.0001$; mean 70 h; table 1).

Multivariate analysis showed male sex as the main predictor of IgE PPM sensitization (adjusted odds ratio, aOR 34.3). Other independent predictors were immedi-

ate discharge from hospital (aOR 12.2) and cutaneous lesion duration < 24 h (aOR 21.4; table 2). Table 3 shows the clinical features and test results of the 48 patients with PPM reactions.

SDS-PAGE and Immunoblotting

IgE immunoblotting showed the presence of several allergens in WL and setae extracts in a wide range of molecular weights. Immunoblotting was positive in 27/48 (56.2%) patients; 21/23 (91.3%) patients had a positive SPT to PPM; 16/27 (59.2%) sera were positive to both extracts and 11/27 (40.7%) only to one extract (3 with WL and 8 patients with setae; fig. 1).

Discussion

This is the first study in a nonhospitalized group of patients investigating differences between sensitized and nonsensitized patients reacting to setae extracts of PPM. Our results showed that involvement of an IgE-mediated hypersensitivity mechanism was common (47.9%) in a group of adults reacting to PPM from an endemic area, and clinical characteristics associated with IgE-mediated hypersensitivity were different from those induced by non-IgE-mediated responses. Moreover, associated risk factors were evaluated, and setae and WL extracts were useful and complementary for the diagnosis of PPM hypersensitivity.

The pathogenic mechanism of PPM-associated rash is not understood entirely. In addition to mechanical irritation, PPM causes hypersensitivity reactions in susceptible individuals [3–10, 14]. There are two different types of skin lesions caused by PPM larvae: immediate wheal-and-flare reactions, and delayed and persistent itchy eruption, usually reported as papular eruption [2, 4, 6–9]. Due to overlapping mechanisms (nonallergic and allergic), it is difficult to assess the clinical significance of each one. Immediate reactions have been explained as result of an IgE-independent degranulation associated with a protein known as thaumetopoein, which is present in the setae of the larva [12]. However, crude larval extracts did not activate basophils from nonsensitized controls, which casts doubt on the part played by fast mediators, such as histamine [10]. On the other hand, several facts support an IgE hypersensitivity mechanism in some of these reactions. (1) Within the same population or workers with similar exposure to *Thaumetopoea* larvae, the great variability among subjects stands out, so that there are patients with almost no symptoms, whereas others present severe reactions [2, 17]. (2) IgE sensitization to PPM larvae has been

Table 1. Distribution of the variables associated with a positive SPT in 48 patients with cutaneous reactions to *T. pityocampa*: bivariate analysis

	Positive SPT, % (total n = 23)	Negative SPT, % (total n = 25)	p value	aOR (95% CI)
Sex				
Male	82.6 (19)	48 (12)	<0.05	5.1 (1.4–19.5)
Female	17.4 (4)	52 (13)		
Age			0.21	
18–35 years	17.4 (4)	40 (10)		0.3 (0.1–1.2)
36–50 years	39.1 (9)	24 (10)		2.0 (0.6–7.1)
>50 years	43.5 (10)	36 (9)		1.4 (0.4–4.4)
Geographic location			0.23	
Urban	17.4 (4)	28 (7)		2.8 (0.9–8.9)
Semi-urban	21.7 (5)	36 (9)		0.5 (0.1–2.2)
Rural	60.9 (14)	36 (9)		0.5 (0.1–1.8)
Exposure				
Daily or weekly	91.3 (21)	60 (15)	<0.05	7.0 (1.3–36.7)
Monthly	4.3 (1)	8 (2)		0.5 (0.0–6.2)
None or very seldom	4.3 (1)	32 (8)		0.1 (0.0–0.8)
Occupational exposure	60.9 (14)	24 (6)	<0.05	0.2 (0.1–0.7)
Atopy	13 (3)	16 (4)	0.084	0.8 (0.2–4.0)
Relatives with PPM reaction	17.4 (4)	12 (3)	0.59	1.5 (0.3–7.8)
Months with symptoms				
June–August	60.9 (14)	28 (7)	<0.05	4.0 (1.2–13.4)
September	47.8 (11)	20 (5)	<0.05	3.7 (1.0–13.1)
October–December	52.2 (12)	16 (4)	<0.05	5.7 (1.5–22.0)
January–May	91.3 (21)	96 (24)	0.50	0.5 (0.0–5.2)
January–December	73.9 (17)	40 (10)	<0.05	4.3 (1.2–14.5)
Number of episodes				
1	0 (0)	16 (4)	<0.05	0.0 (–)
2–5	4.3 (1)	24 (6)		0.1 (0.0–1.3)
>5	95.7 (22)	60 (15)		14.7 (1.7–126.9)
Symptoms				
Urticaria	78.3 (18)	28 (7)	<0.0001	9.3 (2.5–34.7)
Dermatitis	26.1 (6)	68 (17)	<0.005	0.2 (0.0–0.6)
Angioedema	52.2 (12)	12 (3)	<0.005	8.0 (1.9–34.4)
Conjunctivitis	34.8 (8)	24 (6)	0.41	1.7 (0.5–5.9)
Keratitis	8.7 (2)	0 (0)	0.13	–
Rhinitis	30.4 (7)	8 (2)	<0.05	5 (0.9–27.4)
Dyspnea	8.7 (2)	4 (1)	0.5	2.3 (0.2–27.0)
Malaise	8.7 (2)	0 (0)	0.13	–
Affected areas				
Generalized	17.4 (4)	0 (0)	<0.05	–
Neck	91.3 (21)	52 (13)	<0.005	9.7 (1.9–50.4)
Face	47.8 (11)	12 (3)	<0.05	6.7 (1.6–28.9)
Trunk	43.5 (10)	16 (4)	<0.05	4.0 (1.0–15.6)
Extremities	91.3 (21)	84 (21)	0.44	2.0 (0.3–12.1)
Immediate latency (<1 h)	91.3 (21)	32 (8)	<0.0001	22.3 (4.2–119.3)
Duration <24 h	78.3 (18)	24 (6)	<0.0001	11.4 (3.0–44.0)
Emergency department admission	30.4 (7)	8 (2)	<0.05	5.0 (0.9–27.4)

Table 2. Distribution of the variables associated with a positive SPT in 48 patients with cutaneous reactions to *T. pityocampa*: multivariate analysis

	Coefficient of regression (β)	Standard error	aOR (95% CI)	p value
Male sex	3.535	1.513	34.3 (1.8–665.05)	0.019
Immediate latency (<1 h)	2.499	1.190	12.2 (1.2–125.2)	0.036
Duration <24 h	3.066	1.406	21.4 (1.4–337.2)	0.029

Table 3. Clinical characteristics, and SPT and immunoblotting results of 48 adults with cutaneous reactions to PPM (*T. pityocampa*)

No.	Sex	Age, years	Atopic status	Occupational exposure	Skin rash	Other symptoms	SPT area, mm ²		IB	
							WL	setae	WL	setae
1	f	28	R-C-A (pollen, mites)		APD-CPD		–	–	–	–
2	m	22	–		AU-CU		–	–	–	–
3	m	58	–		CPD		–	–	–	+
4	f	86	–	woodcutter	AU-CPD	AE-C-R-D (Ax)	–	–	–	–
5	f	53	–	bricklayer	APD-CPD		13	36	+	+
6	m	58	–		AU-APD	AE-D (Ax)	13	18	+	+
7	m	50	R-C (pollen)		AU		–	–	–	–
8	f	49	–	pinecone collector	AU-CU	AE	20	28	+	+
9	m	53	R-C-A (fungi, dander)		AU, APD	C	–	–	–	–
10	f	48	–	farmer	AU	AE-C-R-D (Ax)	7	78	+	+
11	f	20	–	pinecone collector, forester	CPD	C	–	–	–	–
12	f	61	R-C (pollen)		AU-APD	AE-R	78	95	+	+
13	f	65	–		AU		–	–	–	–
14	f	62	–	bricklayer	AU	AE-R	100	120	+	+
15	m	24	–		APD	C	–	–	–	–
16	f	39	–		APD-CPD	AE-C	–	–	+	–
17	f	73	–	gardener	CPD		–	–	–	–
18	f	23	R-C (pollen, mites, dander)	sports instructor	AU	AE-C-R	36	18	+	+
19	f	72	–		APD		–	–	+	–
20	m	48	–		AU		–	–	–	–
21	m	34	–		CPD		–	–	–	–
22	f	57	–		APD-CPD		–	–	–	+
23	m	17	–		APD-CPD		–	–	–	–
24	f	45	–	pinecone collector	AU-CU	AE	7	28	+	+
25	m	70	–		AU-CU	C-R	7	7	–	+
26	f	64	R-C-A pollen	pinecone and resin collector, woodcutter	AU-CU	C-K	28	–	+	+
27	f	19	R-C pollen, food allergy		APD		–	–	–	+
28	m	45	–		CPD		–	–	–	–
29	m	45	–		AU-ACD		–	–	–	–
30	m	30	–		AU		–	–	–	–
31	m	24	–		AU		20	–	+	–

Table 3. (continued)

No.	Sex	Age, years	Atopic status	Occupational exposure	Skin rash	Other symptoms	SPT area, mm ²		IB	
							WL	setae	WL	setae
32	f	44	–		APD	AE	13	13	+	+
33	f	41	–	forester	AU-APD	AE	70	150	+	+
34	f	65	–	woodcutter	AU-CU		–	160	+	+
35	f	37	–	pinecone collector	AU-CU	AE-C-M	50	45	+	+
36	f	48	–		AU		18	28	–	+
37	m	22	–		AU	C-R	20	–	–	+
38	f	63	–	pinecone collector sand and gravel quarry worker	AU-CU		–	28	–	–
39	f	54	–	pinecone collector	AU-CU	AE-C-K	7	–	–	–
40	f	73	–		APD-CPD		254	1,256	+	+
41	f	35	–	pinecone collector	APD-CPD	AE-C-R-M	–	30	–	+
42	f	48	–		AU-CU-APD-CPD		20	66	+	+
43	f	18	–	pinecone collector	APD-CPD		–	–	–	–
44	m	53	–		AU	C-R	–	–	–	–
45	m	36	–		ACD		–	–	–	+
46	f	80	–		ACD	AE	–	–	–	–
47	f	32	–	gardener	AU		25	28	+	+
48	f	18	–		APD-CPD		–	–	–	–

A = Asthma; APD = airborne papular dermatitis; AU = airborne urticaria; Ax = anaphylaxis; C = conjunctivitis; CPD = contact papular dermatitis; CU = contact urticaria; D = dyspnea; AE = angioedema; f = female; IB = immunoblotting; K = keratitis; M = malaise; m = male; R = rhinitis.

demonstrated by SPT, immunoblotting and ELISA [3–10, 14]. Besides, specific IgG has not been found in these cases, suggesting that PPM setae induce predominantly IgE-mediated immune response in humans [10]. To date, two major allergens from PPM have been described: Tha p 1 from WL [5] and Tha p 2 from setae extracts [11]. (3) Sensitized patients show a significantly higher frequency of immediate and more severe reactions with a shorter duration when compared with nonsensitized subjects [3].

Using setae extracts, this study in nonhospitalized patients showed the same differences as those found previously in patients admitted to an allergy department [3]: SPT-positive patients had more generalized cutaneous reactions (17%) and edema (52%) as well as a shorter latency period (91% <1 h) and duration of cutaneous lesions (78% <24 h) than SPT-negative patients. Moreover, they

had a significantly higher incidence of rhinitis and a higher number of visits to the emergency department. Three subjects had anaphylactic reactions to PPM: specific IgE was detected in 2 cases and in the 3rd case it could not be demonstrated, perhaps because of his old age or because the last reaction occurred many years ago or because of a loss of IgE-mediated responses. In the allergic PPM group, most reactions occurred repeatedly throughout the whole year: although airborne setae have a seasonal pattern, setae can persist longer in the environment and in allergic cases symptoms appear after minimal exposure. Another finding is the higher frequency of cutaneous lesions around the neck among allergic patients, which indicates airborne contamination as the responsible contact mechanism. A prevalence of aeroallergen sensitization was not found in our study population, which is in contrast to IgE

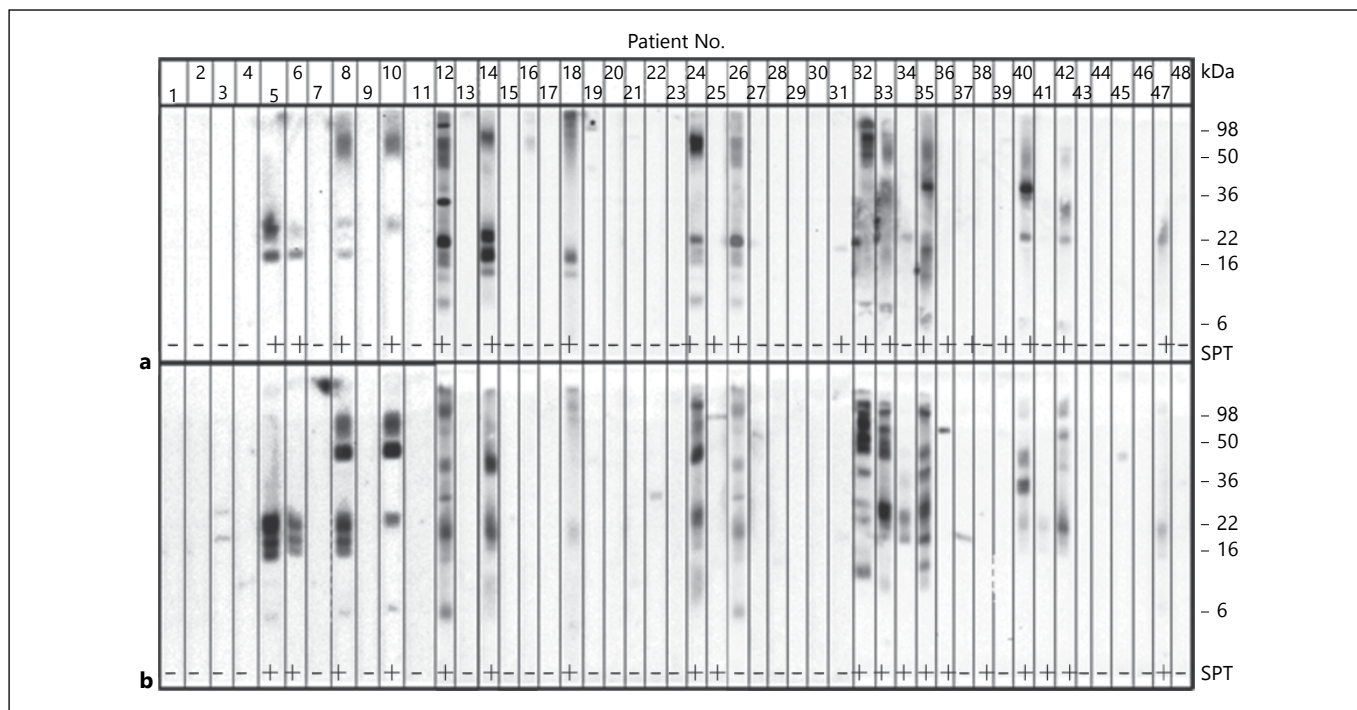


Fig. 1. Allergen detection by IgE immunoblotting. Several allergens are detected by patients in both WL (a) and setae extract (b).

hypersensitivity reactions to other insects such as Hymenoptera [18].

There are few studies on the significance of IgE-mediated mechanisms among nonselected patients reacting to PPM. In patients from a hospital series [3], the prevalence of sensitization is higher (58%) than in this study (48%), probably because patients with severe reactions more often search medical aid and they are more frequently referred to allergy specialists. In children from a rural area, a lower prevalence of sensitization has been found (7%; $n = 60$) [6]. However, this prevalence increases in children from communities located close to infected trees, e.g. peripheral urban areas with higher and repeated exposure (data not shown). In both studies, only WL extracts were used for the diagnosis. However, when WL and setae extracts have been compared by skin testing and immunoblotting, both extracts have demonstrated to be useful and complementary for diagnosis [10]. Our study is the first to demonstrate differences between PPM-sensitized and nonsensitized patients using setae extracts. Standardization of SPT for occupational allergens, such as PPM, is highly recommended [19]. It is a cheap and effective way to determine the presence of specific IgE, and combined with a consistent clinical history enables a confident diagnosis of IgE-mediated allergic diseases [19].

IgE immunoblotting is a less sensitive technique than SPT but it is very specific: 91% of patients with a positive SPT also had positive immunoblotting, demonstrating the usefulness of this test. However, these results should be interpreted cautiously given the modest number of patients, but they are useful for designing future research.

In summary, we showed that almost half of the patients presenting cutaneous reactions to PPM had a positive SPT to setae and WL extracts. In this group, clinical characteristics were different from those induced by non-IgE-mediated mechanisms, such as immediate, repeated and more severe reactions, and symptoms throughout the whole year. Therefore, this insect must be kept in mind as a perennial allergen particularly among workers or residents presenting urticarial eruptions in pine tree areas.

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