

Abbreviations used

BM:	Bone marrow
CLMast:	Instituto de Estudios de Mastocitosis de Castilla La Mancha, The Clinical Reference Centre of the Spanish Network on Mastocytosis (REMA)
ISM:	Indolent systemic mastocytosis
ISMs ⁺ :	Indolent systemic mastocytosis with skin lesions
ISMs ⁻ :	Indolent systemic mastocytosis without skin lesions
MC:	Mast cell
MCAS:	Mast cell activation syndrome
REMA:	Spanish Network on Mastocytosis
sBT:	Serum baseline tryptase
SM:	Systemic mastocytosis
VIT:	Venom immunotherapy
WHO:	World Health Organization

ISMs⁻, especially when IgE-mediated mechanisms are involved. Recently, the Spanish Network on Mastocytosis (REMA) proposed a relatively simple clinical score that proved to be of great value for predicting ISMs⁻ in patients with systemic MC activation symptoms^{14,15}; such score is currently recommended by the REMA in the routine clinical workup of patients presenting with MC activation syndromes (MCASs) suspicious of an underlying clonal MC disorder, in the absence of skin lesions. Furthermore, we have shown that ISMs⁻ patients display clinical, biological, and molecular characteristics that differ significantly from classical ISM presenting with skin lesions (ISMs⁺).¹⁴ Among other features, ISMs⁻ patients show a clearly higher male predominance, lower serum baseline tryptase (sBT) levels, less MC-related symptoms in between acute episodes, and *KIT* mutation typically restricted to MCs. Interestingly, in the latter REMA study,¹⁴ insects (mainly hymenoptera sting) were the most common trigger for acute MC-mediator release episodes in ISMs⁻ patients, with a frequency of up to around one-half of the cases. Because the Instituto de Estudios de Mastocitosis de Castilla La Mancha (CLMast) is a National Reference Centre for Hymenoptera sting anaphylaxis, the overall frequency of SM among patients with insect-induced anaphylaxis could have been overestimated. However, the high frequency of ISMs⁻ in such patients contrasts with that found both in cases presenting with MC activation symptoms triggered by other factors (eg, drugs and foods) and in patients with unidentified triggers. Of note, prospective evaluation of the REMA score has shown a greater sensitivity in patients with anaphylaxis triggered exclusively by insects than in cases associated with other triggers.¹⁴ Altogether, these observations suggest that ISMs⁻ associated with insect-induced anaphylaxis might display unique features that could contribute to explain, at least in part, the previously reported differences between ISMs⁻ and ISMs⁺ cases.

Here, we compared the demographic, clinical, histopathologic, biological, and molecular characteristics of ISMs⁻ patients who experienced anaphylaxis triggered exclusively by insects (n = 143), with other ISMs⁻ cases (n = 72), as well as ISMs⁺ patients who presented with anaphylaxis (n = 56).

METHODS**Clinical and allergic workup**

A total of 335 adult patients (195 men and 140 women; median age, 50 years; range, 18–81 years) with acute systemic MC-mediator release

episodes were studied. From these patients, 271 cases (175 men and 96 women; median age, 48 years; range 18–78 years) were diagnosed with ISM according to the World Health Organization (WHO) criteria.¹⁶ All consecutive patients who gave their informed consent to participate were enrolled in the study from August 1999 to December 2012, at 2 European Reference Centres for mastocytosis: CLMast, Toledo (Spain), and Azienda Ospedaliera Universitaria Integrata, Verona (Italy). Here, we analyzed the data collected prospectively for these patients, from whom 158 have been included in previous reports.^{14,15} In every case, careful skin examination was performed, and baseline MC-related symptoms as well as clinical characteristics of acute MC-mediator release episodes were recorded. For those patients who experienced recurrent MC-mediator release episodes, only the clinical profile of the most severe episode was considered for this study.

Allergic workup systematically included measurement of sBT by ImmunoCAP (Phadia Diagnostics, ThermoFisher Scientific, Uppsala, Sweden) and total serum IgE levels. In addition, a potentially underlying IgE-mediated mechanism for anaphylactic episodes was investigated in suspected subjects through the measurement of specific serum IgE antibodies by ImmunoCAP (ThermoFisher Scientific and CAP-FEIA system Unicap 100; Phadia, Uppsala, Sweden), whenever a specific assay was commercially available; in addition, cutaneous tests (skin prick and intradermal tests) were also performed in patients in whom specific triggers (eg, insect sting, food, or specific drugs such as penicillin) were identified, according to well-established recommendations.¹⁷ In all ISMs⁻ patients who were able to identify wasps or bees as triggers for anaphylaxis and showed both negative specific IgE and cutaneous tests against *Apis mellifera*, *Vespula* species, and *Polistes dominulus* (n = 14), the presence and levels of serum specific IgE antibodies directed against individual components from bee and wasp venoms were assessed. More precisely, specific IgE to 6 natural (n) major allergens (nApi m1, nApi m2, nPol d1, nPol d2, nVes v1, and nVes v5) obtained as described elsewhere¹⁸ were determined with the ADVIA Centaur system (Siemens Medical Solutions Diagnostics, Tarrytown, NY) in 11 patients with a negative allergy study referred to the CLMast; similar determinations (component-based analyses) were performed in 3 Italian patients with a negative allergy study by the ImmunoCAP system (CAP-FEIA system Unicap 100; Phadia) with the use of 3 recombinant (r) allergens (rApi m1, rPol d5, and rVes v5). Specific IgE levels < 0.35 KU/L measured by either the ImmunoCAP or ADVIA systems were considered as negative.

REMA score and BM studies

The REMA score (see Table E1 in this article's Online Repository at www.jacionline.org) was prospectively applied to 279 patients (215 ISMs⁻ and 64 nonclonal MCAS) who underwent a complete BM study because of systemic MC-mediator release symptoms in the absence of skin lesions. The overall score for individual patients ranged from -4 to 7 and a score of ≥2 was considered to be highly predictive for SM, as previously recommended.^{14,15} The sensitivity and the specificity of the REMA score were separately analyzed in cases in whom insect sting was the only trigger identified (n = 159) and in the remaining group of patients (n = 128).

In all cases included in this study, a complete BM study was performed according to previously defined recommendations^{8,16,19} for cytomorphology,^{13,20} immunohistochemistry,^{13,21–23} flow cytometry,^{24–26} and *KIT* mutational analysis.^{6,27,28} In cases studied at the CLMast (n = 271), detection of *KIT* mutations was further performed in genomic DNA from populations purified by fluorescence-activated cell sorting; these populations were BM MCs, neutrophils, eosinophils, monocytes, lymphocytes, CD34⁺ hematopoietic progenitor and precursor cells, and nucleated red cells, as described elsewhere.⁶ Diagnosis of SM was made according to the current WHO criteria.¹⁶

Statistical methods

For all continuous variables, median values and range were calculated, whereas for categorical variables, frequencies were reported. Comparisons between groups were performed with the Mann-Whitney *U* and the χ^2 tests for continuous (nonparametric) and categorical variables, respectively.

P values $\leq .05$ were considered to be associated with statistical significance. All statistical analyses were performed with the SPSS 20.0 statistical software package (SPSS, Chicago, Ill).

RESULTS

Prediction of SM by the REMA score according to the trigger for anaphylaxis

Overall, the REMA score^{14,15} showed both a high sensitivity (91%; 95% CI, 87%-95%) and specificity (75%; 95% CI, 64%-86%), for the identification of SM in the whole group of 279 patients who underwent a BM study because of MC activation symptoms in the absence of skin lesions (Table I). When only those cases presenting systemic MC activation symptoms exclusively triggered by insects ($n = 155$) were considered separately from the other cases ($n = 124$), slight differences were noted between both groups. Such differences consisted of a better sensitivity (92%; 95% CI, 88%-97%) but a lower specificity (67%; 95% CI, 40%-93%) for the insect-only triggered cases than for the other patients with sensitivity of 88% (95% CI, 80%-95%) and specificity of 77% (95% CI, 65%-88%) (Table I).

Triggers for MC-mediator release episodes and ISM patient groups

Overall, insect sting/bite (mainly Hymenoptera sting) was identified as the only trigger for acute MC-mediator release episodes in 143 of 215 ISMs⁻ cases (66%); in the remaining 72 ISMs⁻ patients (34%), other triggers including food ($n = 14$), drugs ($n = 10$), unknown ($n = 9$), stress ($n = 2$), and different combinations of the former triggers ($n = 37$) were recognized (see Fig E1, A and B, in this article's Online Repository at www.jacionline.org). By contrast, MC activation episodes in ISMs⁺ patients ($n = 56$) were mainly triggered by unidentified factors ($n = 21$; 38%), followed by different combinations of triggers ($n = 12$; 21%) and drugs ($n = 11$; 20%), insects being involved as the only trigger for anaphylaxis in only 7 cases (13%) ($P < .001$ vs ISMs⁻) (see Fig E1, C). On the basis of the specific triggers for MC activation episodes identified and the absence versus presence of skin lesions, 3 groups of patients were defined: ISMs⁻ triggered exclusively by insects (insectISMs⁻; $n = 143$), ISMs⁻ triggered by factors other than insects only (otherISMs⁻; $n = 72$), and ISMs⁺ ($n = 56$).

Clinical features of ISMs⁻ cases with insect-induced anaphylaxis compared with other ISM patients

All ISM cases included in the study fulfilled well-defined criteria for anaphylaxis.²⁹ Overall, a significant predominance in men (78% vs 53% and 46%; $P < .001$) together with a lower frequency of recurrent anaphylaxis (45% vs 82% and 71%; $P \leq .001$) were observed among insectISMs⁻ patients compared with both otherISMs⁻ and ISMs⁺, respectively (Table II).

For acute symptoms, significant differences were found among insectISMs⁻ cases versus both otherISMs⁻ and ISMs⁺ cases (Fig 1, A). These included a lower frequency of urticaria (14% vs 32% and 29%; $P = .002$ and $P = .01$, respectively), flushing (12% vs 61% and 43%; $P < .001$), abdominal cramping (4% vs 18% and 32%; $P = .01$ and $P < .001$, respectively), diarrhea (12% vs 31% and 32%; $P = .007$ and $P = .006$, respectively),

TABLE I. Sensitivity and specificity of the REMA score among 279 patients with MCAS in the absence of skin lesions who underwent BM study

	Sensitivity (95% CI)	Specificity (95% CI)
Patients with MCAS exclusively triggered by insects ($n = 155$), %	92 (88-97)	67 (40-93)
Other patients with MCAS ($n = 124$), %	88 (80-95)	77 (65-88)
All patients with MCAS ($n = 279$), %	91 (87-95)	75 (64-86)

headache (3% vs 15% and 18%; $P = .01$ and $P = .005$), and rhinoconjunctivitis (0% vs 16% and 7%; $P = .001$ and $P = .03$, respectively) (Fig 1, A). Interestingly, no significant differences ($> .05$) were found between otherISMs⁻ patients and ISMs⁺ cases for any of these symptoms (Fig 1, A). Noteworthy flushing (6% vs 40% and 63%; $P < .001$) and gastrointestinal symptoms (9% vs 24% and 38%; $P = .004$ and $P < .001$, respectively) outside acute episodes (ie, anaphylaxis) were also significantly less frequent among insectISMs⁻ cases versus both otherISMs⁻ and ISMs⁺ patients, respectively (Fig 1, B). In fact, MC-mediator release symptoms in between anaphylactic episodes were completely absent in 80% of insectISMs⁻ cases and were found in approximately one-half (47%) of otherISMs⁻ ($P < .001$) and in 11% of ISMs⁺ patients ($P < .001$) (Table II). By contrast, no significant differences ($P > .05$) were found among the 3 groups of ISM patients for the presence of organomegalies (9% in insectISMs⁻ vs 8% in otherISMs⁻ and 13% in ISMs⁺ cases) and osteoporosis (29% vs 26% and 16%, respectively) (Table II).

Laboratory features of ISMs⁻ cases with insect-induced anaphylaxis versus other ISM patients

Overall, ISMs⁻ patients showed increased median sBT levels (23.8 $\mu\text{g/L}$; range, 6.4-515 $\mu\text{g/L}$). Interestingly, sBT was significantly lower in insectISMs⁻ cases than in otherISMs⁻ and ISMs⁺ cases: median of 22.4 $\mu\text{g/L}$ (range, 6.4-217 $\mu\text{g/L}$) versus 28.7 $\mu\text{g/L}$ (range, 9.3-515 $\mu\text{g/L}$) and 45.8 $\mu\text{g/L}$ (range, 5.8-241 $\mu\text{g/L}$), respectively ($P = .009$ and $P < .001$, respectively) (Table II). Despite these differences, normal sBT levels ($< 11.5 \mu\text{g/L}$) were detected in a similar proportion of insectISMs⁻ (10/143; 7%), otherISMs⁻ (5/72; 7%), and ISMs⁺ patients (3/56; 5%) (Table II). In turn, the median serum levels of IgE was significantly ($P = .04$) lower among insectISMs⁻ patients (29.3 KU/L; range, 1.7-793 KU/L) versus otherISMs⁻ cases (42.9 KU/L; range, 1-1194 KU/L) but not versus ISMs⁺ patients (27.5 KU/L; range, 1-296 KU/L) (Table II).

Overall, an underlying IgE-mediated mechanism responsible for acute MC-mediator release episodes was found in most ISMs⁻ cases (159/211; 75%); this finding being significantly more frequent ($P \leq .001$) among insectISMs⁻ patients (87%) than among both otherISMs⁻ (51%) and ISMs⁺ (55%) (Table II). Most interestingly, among those 154 ISM cases associated with anaphylaxis triggered by clearly identified wasp or bee sting with ($n = 16$) or without ($n = 139$) additional triggers, 14 patients (9%) had both negative skin and CAP test results, all such cases corresponding to ISMs⁻ patients (Table II). In addition, the median total IgE serum levels of these 14 patients was significantly ($P = .01$) lower than that of patients with IgE-mediated Hymenoptera-induced reactions: 15.9 KU/L (range, 1.7-201 KU/L) versus 37.1 KU/L (range, 5-793 KU/L), respectively.

TABLE II. Clinical and laboratory features of patients with ISMs⁻ associated with insect anaphylaxis (n = 143) versus other ISMs⁻ cases (n = 72) and ISMs⁺ cases associated with anaphylaxis (n = 56)

	OtherISMs ⁻ (n = 72)	P value	InsectISMs ⁻ (n = 143)	P value	ISMs ⁺ (n = 56)
Male, %	53	<.001	78	<.001	46
Median (range) age at onset (y)	38 (13-67)	.007	44 (16-76)	<.001	24 (<1-52)
Recurrent anaphylaxis, %	82	<.001	45	.001	71
MC-mediator release symptoms in between acute episodes, %	53	<.001	20	<.001	89
Organomegalies, %	8	NS	9	NS	13
Osteoporosis, %	26	NS	29	NS	16
Median (range) sBT levels (μg/L)	28.7 (9.3-515)	.009	22.4 (6.4-217)	<.001	45.8 (5.8-241)
sBT levels < 11.5 μg/L, %	7	NS	7	NS	5
sBT levels > 20 μg/L, %	69	NS	62	.01	80
Median (range) serum IgE levels (KU/L)	42.9 (1-1194)	.04	29.3 (1.7-793)	NS	27.5 (1-296)
IgE-mediated anaphylaxis, %*	49	<.001	84	<.001	21
Non-IgE-mediated wasp or bee anaphylaxis, %†	1	NS	9	NS	0

*Overall percentage of patients who showed positive allergy study results determined by CAP and/or skin tests.

†Percentage of patients who were able to clearly identify wasps or bees as triggers for anaphylaxis and showed negative allergy study results confirmed by both CAP and skin tests; in the remaining cases (n = 10; 7%) the allergy study was not available because the culprit insect was not identified. NS, Not significant.

Component-based determination of serum specific IgE antibodies to major Hymenoptera venom allergens among cases with a negative allergy study by means of skin tests and CAP determinations (n = 14) found positive results (>0.35 KU/L) in 7 of 11 patients (64%) who were studied by the ADVIA Centaur system with the use of natural allergens, but none of 3 who were studied by CAP with the use of recombinant antigens (Table III).

WHO criteria for SM among ISMs⁻ patients with insect-induced anaphylaxis versus other ISM patients

All 271 patients with ISM studied strictly fulfilled either the WHO major criterion plus ≥ 1 minor criteria (n = 154/271, 57%) or, in the absence of the major criterion, ≥ 3 minor criteria (n = 117/271; 43%) for SM. Overall, compact BM MC aggregates were less frequently observed among insectISMs⁻ patients versus both otherISMs⁻ and ISMs⁺ (46% vs 70% and 81%, respectively; $P \leq .001$) (Table IV). Similarly, the overall frequency of BM fibrosis was also significantly lower among insectISMs⁻ patients: 15% vs 30% ($P = .01$) and 46% ($P < .001$), respectively (Table IV). By contrast, no significant differences ($P > .05$) were observed among the 3 groups of patients for the frequency of morphologically abnormal, immunophenotypically aberrant, or *KIT*-mutated BM MCs (Table IV). Conversely, insectISMs⁻ patients more frequently ($P < .001$) showed a double BM MC population with coexisting normal (CD25⁻) and aberrant (CD25⁺) MCs (60% of cases) as assessed by flow cytometry than did the other 2 groups (35% of otherISMs⁻ and 16% of ISMs⁺ patients) (Table IV). In addition, multilineage (myeloid or myeloid plus lymphoid) involvement of BM hematopoiesis by the *KIT* mutation was never detected among insectISMs⁻ cases (0/60 cases; 0%), whereas it was present in 5 of 57 cases (9%) with otherISMs⁻ ($P = .02$ vs insectISMs⁻) and in 14 of 53 ISMs⁺ patients (26%) ($P < .001$ vs insectISMs⁻) (Table IV). Despite lower median sBT levels observed among patients with insectISMs⁻ than otherISMs⁻ cases, no statistically significant ($P > .05$) differences were found between both patient groups for the frequency of cases showing sBT levels of >20 μg/L

(62% vs 69%, respectively) (Table II). In contrast, patients with ISMs⁺ more frequently showed sBT levels of >20 μg/L (80%; $P = .01$ vs insectISMs⁻) (Table II).

DISCUSSION

Unlike ISMs⁺ cases, in whom the presence of typical skin lesions usually prompts the study of the BM to confirm the diagnosis of systemic disease, suspicion of mastocytosis in ISMs⁻ may be inconspicuous because patients present with a wide range of heterogeneous MC activation-related symptoms that frequently overlap with those of allergic diseases. This particularly involves patients with ISMs⁻ associated with Hymenoptera venom anaphylaxis, because many of them show hypersensitivity to Hymenoptera venom, sBT levels of <20 μg/L, sometimes in association with increased IgE serum levels.^{10,30,31} Therefore, these cases may be misinterpreted as just presenting an allergic condition with no further investigation being indicated to rule out an associated mastocytosis. Because mastocytosis is a well-known risk factor for adverse reactions to venom immunotherapy (VIT),³¹ the availability of efficient criteria for the identification of SM in patients with MCAS is mandatory.

REMA has proposed¹⁴ and validated¹⁵ a scoring system to predict for SM among patients who present with MC activation symptoms in the absence of skin lesions. This REMA score shows both a high overall sensitivity and specificity, superior to that of sBT.² In the present study, which includes the largest cohort of ISMs⁻ patients reported so far, we further validated the efficiency of the REMA score. Interestingly, our results show a better sensitivity of the REMA score among patients with systemic MC activation symptoms induced only by insects, in line with our previous observations in a significantly smaller patient series.¹⁴ These results indicate a consistent negative predictive value for SM of the REMA score in selecting for those patients with MCAS who have a low probability of an underlying SM and therefore, who might more safely undergo VIT. In turn, differences in the efficiency of the REMA score observed between patients with MCAS triggered exclusively by insects versus other agents also suggest that ISMs⁻ patients with insect-induced anaphylaxis may differ

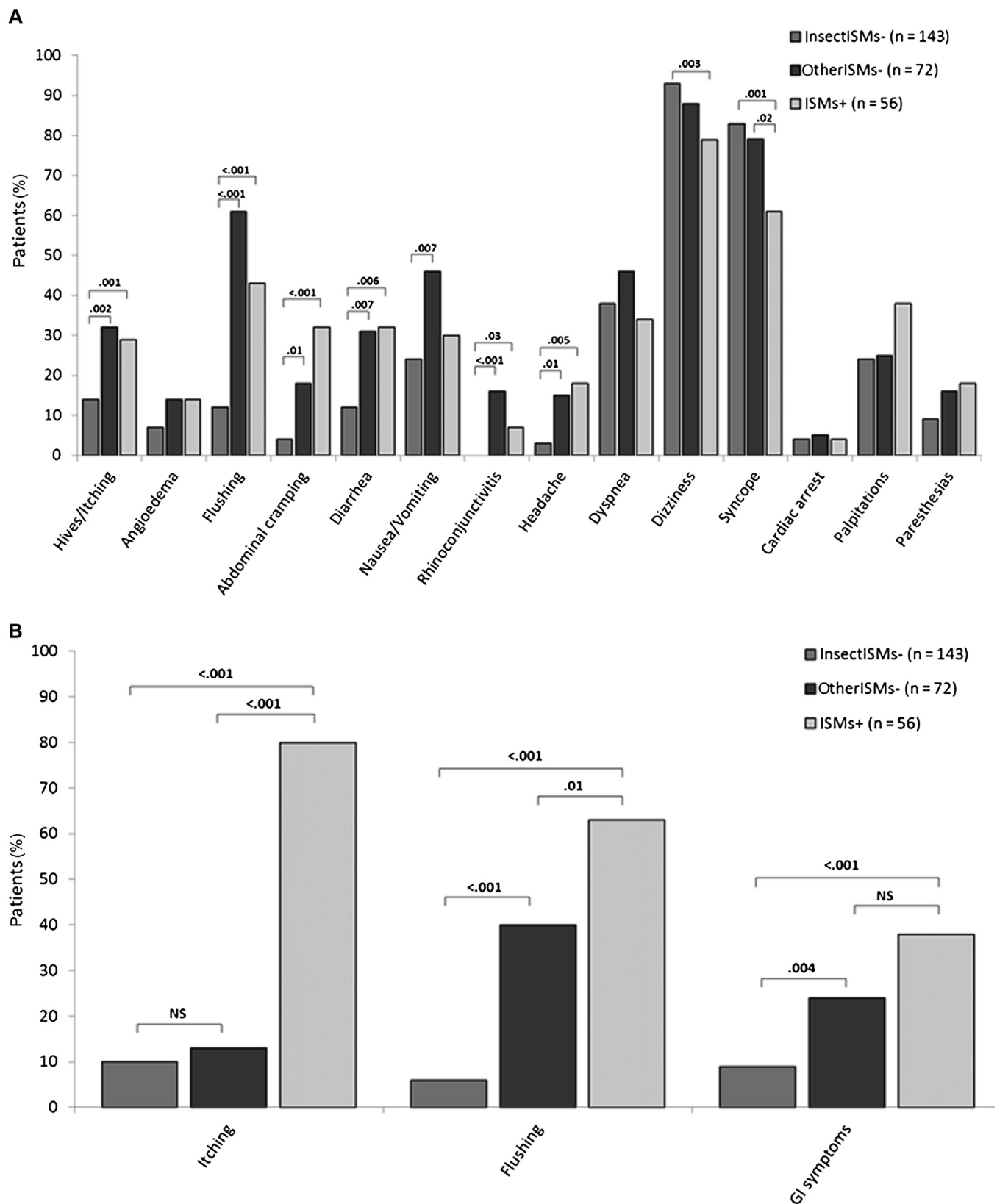


FIG 1. Distribution of clinical symptoms associated with the most severe MC-mediator release episode (**A**) and in between acute episodes (**B**) in patients with insectISM⁻ (n = 143) versus otherISM⁻ cases (n = 72) and ISMs⁺ patients (n = 56). Only clinical symptoms showing statistically significant differences ($P < .05$) among the different groups of patients are shown (**A**). GI, Gastrointestinal; NS, statistically not significant.

TABLE III. Total and specific IgE serum levels from 14 patients with ISMs[−] associated with vespid (wasp or bee)-induced anaphylaxis who showed both negative skin tests and ImmunoCAP analysis by using whole venom extracts

Case ID	Trigger	Total sIgE (KU/L)	Whole venom extracts by CAP (KU/L)			Individual venom components by Advia (KU/L)					
			Apis	Ves	Pol	Api m1	Api m2	Ves v1	Ves v5	Pol d1	Pol d5
SP005	W	27	0.04	0.01	0.09	0.00	0.00	0.00	0.00	0.03	0.00
SP007	W	1.7	<0.10	0.22	0.12	0.13	0.03	0.14	0.12	0.00	0.07
SP021	W	28	0.02	0.03	0.07	0.04	0.00	0.00	0.00	0.88*	0.05
SP025	W	6.2	<0.10	0.12	<0.10	0.00	0.00	0.2	0.5*	0.00	0.00
SP026	W	27	<0.35	<0.35	<0.35	0.02	0.00	0.00	0.53*	0.00	0.09
SP040	W	55	0.04	0.03	0.16	0.00	0.00	0.00	0.00	0.08	0.02
SP042	W	16.8	0.01	0.05	0.29	0.00	0.00	0.11	0.00	1.33*	0.00
SP048	B	201	0.04	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
SP053	W,B	18	0.32	0.00	0.00	0.56*	0.16	0.01	0.03	0.04	0.04
SP057	W	6.3	0.03	0.05	0.18	0.00	0.00	0.00	0.00	0.00	2.09*
SP104	W	5.5	0.01	0.00	0.00	0.00	0.00	0.00	0.85*	0.00	0.00
IT002	W	15	<0.35	<0.35	<0.35	0.00‡	NA‡	NA‡	0.00‡	NA‡	0.03‡
IT019	W	2.1	<0.35	<0.35	<0.35	0.03‡	NA‡	NA‡	0.01‡	NA‡	0.00‡
IT046	B	5.4	<0.35	<0.35	<0.35	0.03‡	NA‡	NA‡	0.00‡	NA‡	0.00‡

Api, *Apis mellifera*; B, bee; NA, not available; Pol, *Polistes dominulus*; Ves, *Vespula*; W, wasp.

*Positive values (>0.35 KU/L).

‡Determined by CAP (KU/L) using recombinant antigens.

TABLE IV. Bone marrow features of patients with ISMs[−] associated with insect anaphylaxis (n = 143) versus other ISMs[−] cases (n = 72) and ISMs⁺ cases associated with anaphylaxis (n = 56)

	OtherISMs [−] (n = 72)	P value	InsectISMs [−] (n = 143)	P value	ISMs ⁺ (n = 56)
Immunohistochemistry					
Compact BM MC aggregates (>15 MCs per cluster), %	70	.001	46	<.001	81
Lymphoid aggregates, %	41	NS	27	<.001	55
Fibrosis, %	30	.01	15	<.001	46
Bone sclerosis, %	2	NS	3	NS	6
Cytomorphology					
>25% of spindled-shaped BM MCs, %	99	NS	96	NS	100
Eosinophilia, %	38	NS	33	NS	35
MEL lesions, %	28	NS	38	NS	51
Dysplasia, %	4	NS	3	NS	7
FCM immunophenotype of BM MCs					
CD25 ⁺ BM MCs, %	100	NS	99	NS	100
Double BM MC population, %	35	<.001	60	<.001	16
Percentage of BM MCs (%), median (range)	0.090 (0.0007-0.99)	NS	0.064 (0.0007-1.0)	.03	0.12 (0.003-1.7)
Percentage of aberrant BM MCs (%), median (range)	0.078 (0.0007-0.99)	NS	0.056 (0.0006-1.0)	NS	0.11 (0.003-1.7)
Molecular markers					
D816V <i>KIT</i> mutation, %	95	NS	96	NS	98
Other <i>KIT</i> mutations, %*	5	NS	2	NS	0
Multilineage <i>KIT</i> mutation, %	9	.02	0	<.001	26

FCM, Flow cytometry; MEL, mast cell plus eosinophil plus lymphocyte; NS, not significant.

**KIT* mutations other than D816V included D816H (1 case in each ISMs[−] group), D816Y (1 case in each ISMs[−] group), and Ins815-816 (1 insectISMs[−] case).

significantly from other ISMs[−] cases, as suggested by our preliminary observations.¹⁴

Previous studies have shown that, beyond the absence of skin lesions, patients with ISMs[−] display unique demographic, clinical, and laboratory characteristics that differ significantly from ISMs⁺ cases. These included a marked male predominance, a lower frequency of MC activation symptoms in between acute episodes, lower sBT levels, a greater frequency of cases with coexisting populations of phenotypically normal and aberrant MC in the BM by flow cytometry, and a lower frequency of multilineage *KIT* mutation.¹⁴ In the present study we show that all these distinctive features between ISMs[−] and ISMs⁺ patients strongly rely on their association with ISMs[−] cases who

presented with anaphylaxis triggered exclusively by insects (insectISMs[−]), but not on ISMs[−] cases associated with other triggers (otherISMs[−]). Male predominance of insectISMs[−] patients may potentially be explained by the increased risk of occupational exposure to insects (eg, beekeepers or farm workers) among the male population because of traditional social roles in rural areas from southern Europe. Such male predominance could also contribute to explain, at least in part, the higher sensitivity, but lower specificity, of the REMA score for the diagnosis of insectISMs[−] versus otherISMs[−] cases, because sex is a relevant factor in this scoring system. In turn, the lower sBT levels observed in this group of patients (compared with otherISMs[−] and ISMs⁺ patients) could be related to a lower total MC burden.

In fact, insectISM⁻ patients also showed a significantly lower frequency of dense compact MC aggregates in BM sections in association with a higher frequency of cases with coexisting CD25⁻ (normal) and CD25⁺ (aberrant) BM MCs and absence of multilineage *KIT* mutation versus the other 2 groups of ISM patients. Altogether, the above findings support the notion that highly sensitive diagnostic methods (eg, multiparameter flow cytometric immunophenotyping and analysis of the *KIT* mutational status on highly purified BM MCs) should be routinely applied in these patients to avoid false-negative results.³² The relatively high frequency of ISMs⁻ patients with insect-induced anaphylaxis carrying sBT of <20 µg/L (ie, more than one-third of cases) is in agreement with previous data¹⁰ and supports the notion that lower sBT cutoff values could be used as a minor diagnostic criterion for SM, at least among these patients. A potential explanation for the lower sBT levels found in insectISM⁻ patients versus otherISM⁻ and ISMs⁺ cases might also be the lower clonal MC load observed in the former group of patients. Alternatively, a greater threshold for chronic nonspecific MC activation could exist in these patients, which is supported by their lower frequency of MC-mediator release symptoms outside anaphylactic episodes and the lower serum levels of total and specific IgE, the latter being undetectable in a significant proportion of cases as discussed further below. In line with this latter hypothesis, insectISM⁻ patients also showed a different clinical profile during acute episodes versus otherISM⁻ and ISMs⁺ cases associated with anaphylaxis, which consisted of a significantly lower frequency of urticaria, rhinoconjunctivitis, headache, flushing, and gastrointestinal symptoms. Whether such clinical differences could be related to the release of distinct profiles of MC mediators remains unknown and deserves further investigations.

Although an overall similar frequency of *KIT* mutation was found in insectISM⁻, otherISM⁻, and ISMs⁺ cases, *KIT* mutation was systematically restricted to the BM MC compartment in the former group of ISMs⁻ patients, whereas multilineage *KIT* mutation was significantly more frequent among otherISM⁻ and ISMs⁺ cases. As previously reported in a large series of SM,^{33,34} this pattern of MC-restricted involvement of hematopoiesis by the *KIT* mutation is associated with a lower tumor burden and confers a particular good prognosis in terms of progression-free survival to ISM patients. Despite all this, a paradox exists between the lower MC tumor load and the frequency of anaphylaxis among insectISM⁻ patients compared with other ISM cases. Although there is no definitive explanation for such apparent discrepancy, the risk of insect-induced anaphylaxis in ISMs⁻ patients could be related to the development of specific IgE against venoms with a high affinity, the direct presence of insect venom in the blood stream, and/or its binding to IgE present on the surface of perivascular mast cells; all these factors may potentially contribute to the development of life-threatening reactions that facilitate early diagnosis of mastocytosis in ISMs⁻ cases. However, further studies are required to elucidate the unique clinical features of this particular group of ISM patients. On top of this, there is a bias in the fact that all patients with insectISM⁻ have been studied because of insect-induced anaphylaxis, whereas the presenting symptom that prompted the study among most of the other SM cases mainly includes the presence of skin lesions and MC-activation symptoms triggered by factors other than insects.

At present, it is well known that an association exists between Hymenoptera anaphylaxis with a negative allergy study and the presence of an underlying SM.^{10,35-37} Among these cases, basophil-activation tests have shown contradictory results.^{38,39} Because of this, alternative mechanisms for antigen-induced anaphylaxis involving non-IgE-mediated MC activation have been postulated; these mechanisms include complement activation,^{40,41} calcitonin gene-related peptide overproduction,⁴² and/or dysfunction of the renin-angiotensin system.^{43,44} Of note, two-thirds of our ISMs⁻ cases associated with Hymenoptera sting anaphylaxis with both negative skin tests and CAP results showed specific IgE against major (natural) allergens with the use of the ADVIA Centaur immunoassay. These results indicate that coexistence of both negative skin tests and specific IgE serum determinations are particularly frequent among ISMs⁻ patients, who usually show low total serum IgE levels; in addition, they point out the potentially greater sensitivity of biotinylated natural allergens subjected to ADVIA analyses (ALK-Abelló, Madrid, Spain) versus recombinant allergens in component-based allergy testing reported by other groups,¹⁸ as well as the need for further prospective studies in larger series of patients in which the ADVIA Centaur analyses are compared with other techniques analyzing extended panels of vespid allergens, including nPol d1 (2/11 positive cases analyzed by the ADVIA system in our series). Altogether, these observations provide evidence about the potential utility of component-based allergy testing methods with the use of natural allergens analyzed by the ADVIA system in patients with ISMs⁻ associated with Hymenoptera anaphylaxis with a negative allergy workup by means of more conventional methods; nevertheless, the true role of component allergens in sensitization deserves definitive demonstration in future prospective studies, because of methodologic differences between the CAP and ADVIA techniques. Because a higher risk of severe (even fatal) reactions exists among untreated patients with SM with history of Hymenoptera venom anaphylaxis,^{10,45-47} and VIT is an efficient therapeutic approach with low toxicity in SM,^{31,48-50} it seems reasonable to consider evaluating such therapy in this subgroup of patients within future prospective clinical trials.

In summary, the results here presented indicate that previously reported demographic, clinical, and laboratory differences between ISMs⁻ and ISMs⁺ are most likely due to the association of such features with ISMs⁻ cases with insect-induced anaphylaxis. Therefore, compared with ISMs⁺ patients as well as with other ISMs⁻ patients, ISMs⁻ cases associated with insect-induced anaphylaxis show a clear male predominance in association with a unique pattern of MC-related symptoms, and a low tumor burden, as reflected by lower sBT levels, a lower frequency of dense multifocal BM MC aggregates, increased coexistence of immunophenotypically aberrant and normal MCs in the BM, and systematic absence of multilineage involvement of hematopoiesis by the *KIT* mutation. Altogether, these findings indicate that ISMs⁻ associated with insect-induced anaphylaxis may represent a unique clinical subtype of SM, with a remarkably low MC load that requires usage of the most sensitive and specific diagnostic techniques for the immunophenotypic and molecular analysis of BM MCs, to confirm the clonal nature of the disease. In addition, our results also suggest that true Hymenoptera sensitization can be demonstrated by the measurement of specific IgE against venom components in a significant proportion of cases who appear to be non-IgE

mediated by means of conventional methods, opening a door for evaluating the potential benefits of VIT in this subgroup of ISMs⁺ cases.

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Clinical implications: Indolent systemic mastocytosis without skin lesions (ISM⁻) associated with insect-induced anaphylaxis displays unique clinical and biological features that differ significantly from features found in other ISMs⁺ patients and patients with ISM with skin lesions.

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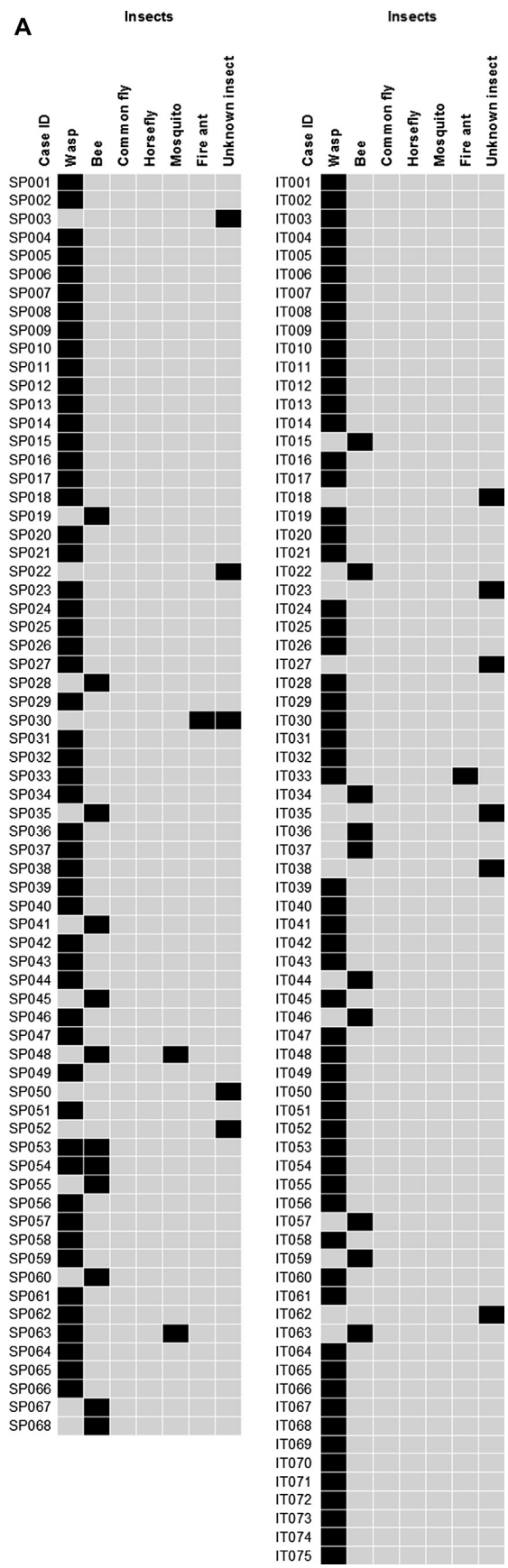
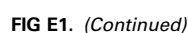
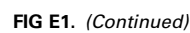


FIG E1. Distribution of different triggers involved in acute MC-mediator release episodes in patients with insectISM⁻ (n = 143) **(A)**, otherISM⁻ (n = 72) **(B)**, ISMs⁺ (n = 56) **(C)**, and nonclonal MCAS (n = 64) **(D)**. *Black boxes* indicate the specific trigger(s) for MC-mediator release episodes involved in every case, and *striped boxes* represent those fish-triggered cases in which hypersensitivity to *Anisakis simplex* was found by CAP and/or skin tests.





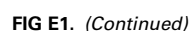


TABLE E1. Clinical and biochemical criteria used by the REMA score to predict for bone marrow mast cell clonality and systemic mastocytosis in patients who present with systemic mast cell-mediator release symptoms in the absence of skin lesions

Variable	Score
Gender	
Male	+1
Female	−1
Clinical symptoms	
Absence of urticaria, pruritus and angioedema	+1
Urticaria, pruritus and/or angioedema	−2
Presyncope and/or syncope	+3
Serum baseline tryptase	
<15 ng/mL	−1
>25 ng/mL	+2

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