doi: 10.1111/j.1365-2222.2009.03210.x
Clinical & Experimental Allergy, **39**, 717–725

ORIGINAL ARTICLE **Clinical Allergy**

Cutaneous manifestations in Hymenoptera and Diptera anaphylaxis: relationship with basal serum tryptase

A. Potier*, C. Lavigne†, D. Chappard‡, J. L. Verret§, A. Chevailler¶, B. Nicolie* and M. Drouet*

**Department of Allergology, Angers University Hospital, Angers, France, †Department of Internal Medicine and Gerontology, Angers University Hospital, Angers, France, ‡INSERM, U922, Faculty of Medicine, Angers University Hospital, Angers, France, §Department of Dermatology and Venereology, Angers University Hospital, Angers, France and ¶Immunology and Allergology Laboratory, Angers University Hospital, Angers, France*

Clinical & Experimental Allergy

Summary

Objectives To compare the clinical presentation of systemic anaphylaxis to Hymenoptera and Diptera with regard to basal serum tryptase (BT) and to evaluate mastocytosis in patients with elevated tryptase.

Patients and Methods The medical records of 140 patients with a history of a systemic reaction to venom were retrospectively reviewed. Symptoms and severity of anaphylaxis and BT were recorded. Most patients with elevated tryptase were screened for mastocytosis: a dermatological examination with a skin biopsy was performed in 19 cases and a bone marrow biopsy in 14 cases.

Results Tryptase was elevated in 23 patients. These patients reported fewer usual skin reactions (urticaria in 26.1% of cases with raised tryptase vs. 76.1% of cases with normal tryptase), more flushing (52.2% vs. 4.3%) and frequently did not present skin reaction (26.1% vs. 9.4%). They presented a more severe reaction (mean grade of severity: 3.48 vs. 2.69). Mastocytosis was diagnosed in seven patients with elevated tryptase: indolent systemic mastocytosis in six cases and cutaneous mastocytosis without systemic involvement in one case. In five cases, mastocytosis was previously undiagnosed. Lesions of cutaneous mastocytosis, diagnosed in five patients, consisted of urticaria pigmentosa in all cases and were often inconspicuous.

Conclusion These results demonstrate particular clinical features of the allergic reaction in patients with elevated BT and the higher frequency of mastocytosis in this population. In patients with a severe anaphylactic reaction without urticaria, but with flushing, tryptase should be assayed and an underlying mastocytosis should be considered.

Keywords anaphylaxis, cutaneous mastocytosis, insect allergy, systemic mastocytosis, tryptase
Submitted 10 September 2008; revised 15 December 2008; accepted 1 January 2009

Correspondence:
Dr Martine Drouet, Unité d'Allergologie Générale, Centre Hospitalo-Universitaire, 4 rue Larrey, 49933 Angers, Cedex 9, France.
E-mail: madrouet@chu-angers.fr
Cite this as: A. Potier, C. Lavigne, D. Chappard, J. L. Verret, A. Chevailler, B. Nicolie and M. Drouet, *Clinical & Experimental Allergy*, 2009 (39) 717–725.

Introduction

The association between systemic reactions to Hymenoptera stings and mastocytosis has been occasionally described for many years [1–9]. Mastocytosis is a rare and heterogeneous disorder characterized by abnormal accumulation of mast cells (MC) in one or more organs. The symptoms and signs of the disease may be due to the release of MC mediators or organ infiltration.

The introduction of a method to determine MC tryptase provided a new approach to the diagnosis of mastocytosis [10–12]. This serine protease, stored almost exclusively in the secretory granules of MC, is found to be elevated after MC activation such as anaphylaxis but is also correlated with the cumulative MC burden [10–12]. A consensus conference adopted by the World Health Organization proposed a persistently elevated tryptase level as a minor criterion for the diagnosis of systemic mastocytosis [13–15].

Recently, several authors have reported the frequent occurrence of constitutively elevated tryptase in patients with severe sting reactions [16] and diagnosed mastocytosis in some of these patients [17, 18].

The symptoms of the sting reaction with regard to basal tryptase level were studied in 140 patients with systemic anaphylaxis to Hymenoptera and Diptera, and patients with elevated tryptase were screened for mastocytosis.

Patients and methods

Patients

The medical records of 140 patients with a history of a systemic reaction to Hymenoptera or Diptera were retrospectively reviewed. All patients ($N = 131$) who had started immunotherapy in the Department of Allergology between April 2002 and May 2005 were included. All patients ($N = 9$) with elevated tryptase seen during the same period for follow-up of systemic venom allergy were also included.

Retrospective analysis of the anaphylactic reaction to insect

Cutaneous, respiratory and digestive symptoms of the systemic reaction were recorded. Based on clinical symptoms, the severity of the systemic reaction was classified into four grades: grade 1 (generalized skin reaction, angio-oedema, and discomfort without loss of consciousness), grade 2 (nausea and vomiting, diarrhoea, and abdominal pain), grade 3 (dysphagia, dysphonia, and dyspnoea) and grade 4 (loss of consciousness, hypotension, collapse, or shock). In patients with recurrent anaphylaxis, the most severe reaction was analysed.

Estimation of basal serum tryptase and total immunoglobulin E

Serum tryptase level was measured after an anaphylaxis-free interval (at least 4 weeks after the anaphylactic reaction) by a commercial fluoro enzyme immunoassay (UniCAP Phadia, Uppsala, Sweden): concentrations $\geqslant 13.5$ ng/mL were considered to be elevated and were confirmed on another serum sample when possible. Total IgE was also assayed (UniCAP Phadia): this result was not available for one patient with normal tryptase. The relevant insect was identified by anamnestic data, skin tests, IgE evaluation, and a basophil activation test (BAT). The BAT is usually performed in each case of reaction to venom in the Department of Allergology [19].

Screening for mastocytosis in patients with elevated tryptase

Patients with elevated basal serum tryptase (BT) were screened for mastocytosis. A history of systemic symptoms unrelated to insects, consistent with MC mediator-related symptoms, was recorded.

A dermatological examination was performed and a skin biopsy using a non-epinephrine local anaesthetic was taken from a skin lesion suggestive of mastocytosis or from normal skin in the case of a negative examination. Histologic examination was performed by HES and Alcian Blue permanganate staining. Cutaneous mastocytosis was defined by the presence of a MC infiltrate in the dermal tissue [20]. In the case of a sparse infiltrate, the numerical criterion of more than 10 MC per field using $\times 40$ objective magnification was applied [21].

Systemic involvement was evaluated on bone marrow and bone biopsies. Samples were obtained from the same iliac crest. Bone marrow biopsies were fixed, decalcified, and paraffin-embedded according to standard techniques. Bone biopsy was performed with a 7 mm Meunier's trephine (Commeca SA, Beaucouzé, France), fixed in an ethanol–formalin solution and embedded in a polymethylmethacrylate polymer without decalcification. Standard HPS, Giemsa, and Toluidine Blue staining for the bone marrow specimen, and Goldner and Toluidine Blue staining for the bone specimen were performed. Immunohistochemistry using monoclonal antibodies for tryptase, c-kit and CD20 was also performed. According to established consensus criteria, the diagnosis of systemic mastocytosis was defined by the presence of one major criterion [dense (> 15) multifocal infiltrate of MC] and one minor criterion, or three minor criteria [predominance ($> 25\%$) of spindle-shaped MC in the infiltrate, c-kit point mutation at codon 816 and persistent serum total tryptase concentration > 20 ng/mL] [14, 15]. The fourth minor criterion (co-expression of CD2 and/or CD25 by kit+MC) was not studied, as bone marrow flow cytometry for this parameter is not performed routinely in our hospital. Mastocytosis was then subclassified according to the WHO classification [15].

Statistics

Statistical analyses were performed using SPSS for Windows version 15.0. They included frequency distribution and percentages. Differences between groups were compared using χ^2 and Mann–Whitney or Student tests, as appropriate. Differences were considered significant when two-sided P-values were < 0.05.

Results

Basal serum tryptase

BT was elevated in 23/140 patients included in this study (range: 14.3–156 ng/mL), with a mean of 33.3 ng/mL (Table 1). BT was normal in 117 patients (range: 1.0–13.2 ng/mL), with a mean of 5.5 ng/mL. Among patients with elevated tryptase values ranged from 13.5 to 20 ng/mL for eight patients and were > 20 ng/mL for 15 patients. To study the frequency of elevated tryptase, only the population of 131 patients included with the same criterion (immunotherapy initiated during the study period) was considered: an elevated tryptase was observed in 14 (10.7%) cases.

Table 1. Demographic data, insect responsible, total IgE and serum tryptase levels in 140 patients with regard to basal serum tryptase (BT)

Patient group	Normal BT	Elevated BT
N	117	23
Age: mean value (range)	44.4 (9–80)	51.1 (29–85)
Gender: male/female	88/29	18/5
Insect responsible:		
Hymenoptera (Vespidae/honeybee/Vespidae and honeybee)	96/19/1	20/1/1
Diptera (horse fly/horse fly and mosquito)	1/0	0/1
Total IgE (kIU/L): mean value (range)	218 (15–2692)	99 (7–447)
BT (ng/mL): mean value (range)	5.5 (1.0–13.2)	33.3 (14.3–156)

Patients

Of the 117 patients with normal tryptase, 29 (25%) were females and 88 (75%) were males (male-to-female ratio: 3 : 1) (Table 1). A similar male predominance was observed in the elevated BT group, with five (22%) females and 18 (78%) males (male-to-female ratio: 3.6 : 1). The mean age was 44.4 years in patients with normal tryptase (range: 9–80 years) and 51.1 years in patients with elevated BT (range: 29–85 years). Among the study population of 140 patients, 138 were allergic to Hymenoptera venom: 116 to Vespidae (Vespula or Vespa), 20 to honeybee, and two to Vespidae and honeybee. Two patients were allergic to Diptera: one to horse fly (Tabanus sp) and one to mosquito and horse fly. None of the patients with normal tryptase reported a history of mastocytosis, whereas two patients with elevated BT had been previously diagnosed with indolent systemic mastocytosis (ISM): one patient had bone marrow involvement with osteoporosis (case 6, Table 3) and another patient had bone marrow involvement with cutaneous mastocytosis of the urticaria pigmentosa (UP) type (case 12, Table 3). the total IgE level was lower in patients with elevated BT (mean value: 99 kIU/L) than in cases with normal tryptase (218 kIU/L), but this difference was not statistically significant.

Clinical presentation of the systemic reaction to the insect sting

These results are summarized in Table 2.

Analysis of cutaneous symptoms did not include local or regional reactions. Six (26.1%) of the 23 patients with elevated tryptase did not report any generalized skin reaction, compared with only 11/117 (9.4%) in patients with normal BT ($P = 0.037$). Usual skin reactions (urticaria, angio-oedema, and generalized pruritus) were more frequently observed in patients with normal BT (88.9%), than in those with elevated tryptase (52.2%) ($P < 0.001$). In particular, urticaria was observed in the vast majority of patients with normal tryptase (76.1%), but in only six (26.1%) patients with elevated BT ($P < 0.001$). In contrast, patients with elevated tryptase frequently reported other skin symptoms, such as flushing (52.2%), compared with only 4.3% of patients with normal BT ($P < 0.001$). In patients with tryptase > 20 ng/mL, urticaria (20%) was less frequent and flushing (60%) was more common, compared with patients with elevated BT between 13.5 and 20 ng/mL (urticaria: 37.5%, flushing: 37.5%); however, the small sample sizes of subgroups did not allow statistical analysis of this correlation.

Digestive symptoms were more common in patients with elevated tryptase and respiratory reactions were less frequent; however, these differences were not statistically significant.

The mean grade of severity was higher in patients with elevated tryptase (3.48) than in patients with normal BT (2.69) ($P = 0.006$). 82.6% of patients with elevated BT experienced grade 4 reactions, compared with only 37.6% of patients with normal tryptase. Grade 1 reactions occurred in a minority of patients with elevated tryptase (17.4%), but in 32.5% of patients with normal tryptase. Grade 4 reactions occurred more frequently in patients with tryptase > 20 ng/mL (86.7%) than in patients with BT between 13.5 and 20 (75%), but the small sample sizes of subgroups did not allow statistical analysis of this correlation.

The number of allergic reactions to venom for each patient ranged from 1 to 4 regardless of the basal tryptase value. The most frequent situation was a single reaction for 88 (75.2%) patients with normal tryptase and 10 (43.5%) patients with elevated BT. The mean number of allergic reactions was higher for patients with elevated tryptase (1.78) than for patients with normal BT (1.28) ($P = 0.012$).

Screening for mastocytosis in patients with elevated tryptase

The characteristics of patients with elevated BT are summarized in Table 3. Four of the 23 patients with elevated BT were not reviewed: patient 6 had already been diagnosed for ISM with regular follow-up; patient 7 had a history that was not suggestive of mastocytosis and only slightly elevated BT; and patient 14 refused further screening; patient 21 was considered to be too old for systemic screening (85 years).

Screening for cutaneous mastocytosis. Cutaneous mastocytosis (CM) was diagnosed clinically in 5/19 patients (Table 4). CM had been previously diagnosed in only one case (case 12). The type of CM was UP in all cases, characterized by red-brown macular small lesions with a symmetrical distribution. Few lesions were observed in three patients (cases 9, 15, and 23). Darier's sign was found in three out of five patients (cases 15, 19, and 23). The lesions mainly involved the trunk in five cases and

Table 2. Clinical symptoms, severity and number of allergic reactions with regard to basal serum tryptase (BT)

			Elevated BT					
Patient group	Normal BT		All patient with elevated BT		Subgroup $13.5 \leqslant BT \leqslant 20$		Subgroup $BT > 20$	
N	117	100%	*N*: 23	100%	*N*: 8	100%	*N*: 15	100%
Usual skin reaction	104	88.9%	12	52.2%	5	62.5%	7	46.7%
Pruritus without urticaria	9	7.7%	3	13.0%	1	12.5%	2	13.3%
Urticaria	89	76.1%	6	26.1%	3	37.5%	3	20%
Angio-oedema	55	47.0%	5	21.7%	2	25.0%	3	20%
Flushing	5	4.3%	12	52.2%	3	37.5%	9	60%
No skin reaction	11	9.4%	6	26.1%	1	12.5%	5	33.3%
Digestive reaction	26	22.2%	7	30.4%	1	12.5%	6	40%
Nausea, vomiting	15	12.8%	6	26.1%	1	12.5%	5	33.3%
Diarrhoea	2	1.7%	0	0%	0	0%	0	0%
Abdominal pain	5	4.3%	3	13%	0	0%	3	20%
Dysphagia	7	6%	0	0%	0	0%	0	0%
Respiratory reaction	42	35.9%	7	30.4%	3	37.5%	4	26.7%
Dysphonia	5	4.3%	0	0%	0	0%	0	0%
Laryngeal dyspnoea	4	3.4%	1	4.3%	0	0%	1	6.7%
Bronchial dyspnoea	3	2.6%	0	0%	0	0%	0	0%
Unspecified dyspnoea	32	27.4%	6	26.1%	3	37.5%	3	20%
Grade of severity								
Grade 1	38	32.5%	4	17.4%	2	25%	2	13.3%
Grade 2	4	3.4%	0	0%	0	0%	0	0%
Grade 3	31	26.5%	0	0%	0	0%	0	0%
Grade 4	44	37.6%	19	82.6%	6	75%	13	86.7%
Mean grade	2.69		3.48		3.25		3.6	
Number of allergic reactions								
1 reaction	88	75.2%	10	43.5%				
2 reactions	26	22.2%	9	39.1%				
3 reactions	2	1.7%	3	13.1%				
4 reactions	1	0.9%	1	4.3%				
Mean number of reactions	1.28		1.78					

were disseminated in one case (case 19). Only two patients were symptomatic and complained of pruritus, caused by hot water in one patient (case 9) and with no triggering factor in another patient (case 12). BT was higher than 20 ng/mL in four out of five patients.

Histologic examination of skin biopsy revealed a MC infiltrate accumulating in the dermal tissue in three patients. Skin biopsy of a scraped lesion was performed in one patient (case 23): histologic examination only evidenced a lymphoplasmocytic infiltrate with negative Alcian Blue permanganate staining, but this could be explained by MC degranulation due to skin friction. However, as skin lesions with Darier's sign were consistent with mastocytosis and as systemic mastocytosis was also demonstrated, this patient was considered to present cutaneous mastocytosis. A skin biopsy was not performed in one patient (case 12), as this patient presented multiple, persistent skin lesions with systemic mastocytosis.

Among the remaining 14 patients, dermatological examination was normal for 11 patients and a skin biopsy on healthy skin was also normal in 10 of these 11 patients. In three cases, a skin biopsy of atypical red or telangiectatic macular lesions showed a non-specific inflammatory infiltrate with negative Alcian Blue permanganate staining.

Screening for systemic mastocytosis. Among the 19 patients evaluated for mastocytosis, 14 were investigated for systemic involvement. A bone marrow biopsy was not performed in three patients in whom the history was not suggestive of systemic mastocytosis (typical, mild allergic reaction, absence of cutaneous mastocytosis, normalized or slightly elevated tryptase) and this investigation has not yet been performed in two other patients (nos. 3 and 11).

Systemic mastocytosis (SM) was detected in five out of 14 patients (Table 4). SM had previously been diagnosed in only one patient (case 12). In every case, bone biopsies showed a typical dense multifocal MC infiltrate with spindle-shaped cells, whereas bone marrow biopsies showed a typical infiltrate only in case 19 (uncontributive in case 23, normal in case 12, and increased number of MC without aggregate formation in cases 10 and 15). All but one of these patients presented cutaneous mastocytosis.

Table 3. Characteristics of patients with elevated basal serum tryptase (BT)

N	Gender	Age	Number of reactions to venom	HP/DP	Symptoms of the allergic reaction	Grade of severity	BT	History of mastocytosis	Evaluation for mastocytosis
1	M	43	2	V	F, D, R, C	4	18.5	–	D
2	M	37	4	V, B	U, R, C	4	16.9	–	D
3	M	46	3	V	C	4	23.2	–	D
4	M	42	1	V	C	4	21.3	–	D
5	M	71	1	V	C	4	17.2	–	D
6	M	55	1	V	F, R, C	4	113	ISM	ND
7	F	49	1	V	F, AO, DC	1	14.3	–	ND
8	M	35	1	V	F, AO, D, C	4	28.5	–	D
9	M	29	2	B	F, D, C	4	23.3	–	D
10	F	45	2	V	P, F, D, C	4	20.6	–	D
11	M	55	1	V	C	4	21	–	D
12	M	35	1	V	F, D, C	4	156	ISM with CM	D
13	F	59	2	V	F, AO, R, C	4	26.4	–	D
14	M	69	1	V	U, DC	1	22.6	–	ND
15	M	59	3	V	C	4	21.4	–	D
16	M	27	2	V	U, C	4	16.6	–	D
17	F	68	2	V	F, C	4	19.8	–	D
18	M	41	3	H, M	F, P, AO, D, R, C	4	65	–	D
19	F	47	2	V	D, R, C	4	22.9	–	D
20	M	69	1	V	F, U, DC	1	24.7	–	D
21	M	85	1	V	F, U, C	4	35.3	–	ND
22	M	54	2	V	U, AO	1	19.9	–	D
23	M	33	2	V	P, R, C	4	18.2	–	D

N, patient; M, male; F: female; HP/DP, Hymenoptera/Diptera; V, Vespidae; B, honeybee; H, horse fly; M, mosquito; F, flushing; D, digestive symptom; R, respiratory symptom; C, collapse, shock or loss of consciousness; U, urticaria; DC, discomfort; AO, angio-oedema; P, isolated pruritus; ISM, indolent systemic mastocytosis; CM, cutaneous mastocytosis; D/ND, done/not done.

All patients were classified as presenting indolent SM; one patient without cutaneous mastocytosis (case 10) was subclassified as bone marrow mastocytosis (a subgroup of ISM). Tryptase was >20 ng/mL in four of these five patients.

In five patients, bone marrow or bone biopsies showed an increased number of MC with a diffuse infiltrate often composed of spindle-shaped cells with a perivascular or a paratrabecular arrangement. However, the diagnosis of SM could not be confirmed, as this infiltrate did not form aggregates and not all of the consensus criteria were satisfied. Cutaneous mastocytosis was diagnosed in only one case.

Screening for systemic mastocytosis was negative for four patients. None of these patients presented cutaneous mastocytosis and two of them had slightly elevated tryptase (between 13.5 and 20 ng/mL).

Mast cell mediator-related symptoms. Systemic symptoms unrelated to insect sting and consistent with MC mediator-related symptoms occurred in eight out of 19 patients (Table 4). Flushing was the most common symptom, reported by 5/8 patients. Severe reactions (discomfort, loss of consciousness, hypotension, collapse or shock) occurred in three out of cases. Other symptoms included digestive symptoms in two cases, urticaria in one case, rash in one case and respiratory symptoms in one case. Medications and alcohol/food were identified as the two main causative factors, each occurring in four cases: anti-inflammatory drugs were suspected in two cases and three patients complained of alcohol intolerance. In two patients, the causative factor appeared to be stress or asthaenia. No triggering factor was identified in two cases. Four of the six patients with mastocytosis reported such systemic reactions: flushing was the most common symptom and medication was the main triggering factor.

Discussion

Tryptase is a serine protease, selectively produced by MC. The commercially available fluoroimmunoenzymatic assay measures total tryptase, i.e. mature and precursor forms of α and β tryptase [12, 22]. Total tryptase is elevated in the case of sufficiently severe systemic anaphylaxis but is also correlated with total MC burden, especially in mastocytosis [10, 12]. Elevated BT has been detected in various other haematologic disorders, particularly in myeloproliferative and myelodysplastic disorders and has also been reported in patients with haemodialysis end-stage kidney disease [23] and in cutaneous

Table 4. Screening for mastocytosis in 19 patients with elevated basal serum tryptase (BT)

		SM					MC mediator-related symptoms	
		Conclusion	Major criterion	Minor criteria				
n	CM		Multifocal dense infiltrate of MC in bone marrow	>25% spindle-shaped MC in infiltrate	c-kit mutation	BT >20	Symptoms	Causative factor
1	–	ND				–		
2	–	ND				–		
3	–	ND				–		
4	–	MC Infiltrate	–	+	ND	+	–	
5	–	MC Infiltrate	–	+	ND	–	+	F, R, C	0, mammal's meat
8	–	MC Infiltrate	–	+	–	+	+	F, D	0, stress
9	UP	MC Infiltrate	–	+	ND	+	+	F, DC	Local anesthesia with lidocaine
10	–	ISM	+	+	ND	+	–		
11	–	ND					–		
12	UP	ISM	+	+	ND	+	+	F, D	AI drug, codeine, iodinated contrast media, stress, strawberry, alcohol
13	–	–	–	–	ND	+	–		
15	UP	ISM	+	+	ND	+	+	U	Alcohol
16	–	–	–	–	ND	–	–		
17	–	–	–	–	ND	–	+	F	Hydroxyzine, antidepressant
18	–	MC Infiltrate	–	+	–	+	+	C	Alcohol
19	UP	ISM	+	ND	ND	+	+	Rash	Acetylsalicylic acid
20	–	–	–	–	ND	+	–		
22	–	ND					–		
23	UP	ISM	+	+	ND	–	–		

CM, cutaneous mastocytosis; UP, urticaria pigmentosa; SM, systemic mastocytosis; MC, mast cell; ISM, indolent systemic mastocytosis; ND, not done; F, flushing; R, respiratory reaction; C, collapse or shock; D, digestive reaction; DC, discomfort; U, urticaria; AI drug, anti-inflammatory drug.

mastocytosis with extensive and diffuse lesions [12, 24–26]. A persistently elevated tryptase level is recommended by the World Health Organization as a minor criterion in the diagnostic evaluation of systemic mastocytosis after exclusion of these other conditions [14, 15]. Elevated basal tryptase was observed in 10.7% of patients of the present study population, a fairly similar, although slightly higher frequency than that reported previously [16–18]. Raised tryptase was significantly associated with a greater severity of the allergic reaction in our study population. In line with the literature, elevated tryptase can be considered to be a risk factor for severe allergic reactions to Hymenoptera stings [16–18].

The cutaneous manifestations of the allergic reaction differed significantly in patients with elevated tryptase. Usual skin reactions, especially urticaria, were less frequent in patients with raised BT. Moreover, the absence of cutaneous symptoms was commonly observed in this group. On the contrary, flushing was frequently reported by patients with elevated BT. Due to the retrospective nature of this study, clinical information on flushing, particularly in the population with normal tryptase, may be lacking. Nevertheless, according to our clinical experience and published data, urticaria and/or angio-oedema are the most common symptoms of anaphylaxis, reported in more than 85%, whereas flushing is quite rare, occurring in about 25% of cases [27, 28]. This cutaneous feature has not yet been correlated with regard to basal tryptase but was recently described in patients with mastocytosis: in a review of anaphylaxis in 120 patients with mastocytosis (including 74 adults: 13 CM and 61 SM), flushing and pruritus were common in adults (33% and 14% respectively), compared with urticaria (17%) and angio-oedema (11%) [29]. Similarly, Koterba compared the clinical presentation of idiopathic anaphylaxis in 30 patients including 15 cases of ISM, and found no cases of urticaria among patients with mastocytosis vs. 33% in the other group: they concluded that urticaria was a negative clinical indicator for the presence of MC disease in patients with anaphylaxis [30]. Few data are available specifically concerning the skin reactions to venom and serum tryptase levels, as cutaneous symptoms are frequently not reported in papers on Hymenoptera reactions in patients with mastocytosis [3–5] or elevated BT [8, 17, 18, 31–33]. However, skin reactions appear to be less frequent in most cases with elevated tryptase [6, 9]. No definite explanation can be given for this particular clinical presentation. In venom-allergic patients with

severe cardiovascular symptoms, it has been speculated that the cardiovascular system could be predominantly affected because of a fast mediator release. Consequently, the release of epinephrine to control the circulation could lead to a constriction of the peripheral blood vessels, and possibly to an inhibition of skin symptoms [34].

In this study population, patients with elevated tryptase often presented lower total IgE levels, although no statistically significant difference was demonstrated. In the same way, Sturm et al. [34] have recently demonstrated that the severity of sting reactions was influenced by total IgE levels and that patients with severe reactions showed significantly lower total IgE levels. Moreover, low total and specific IgE have been frequently observed in previous case reports of patients with mastocytosis [1, 6, 30, 35] or with elevated tryptase [16]. This phenomenon may be explained by increased absorption of circulating IgE by abundant tissue MC. In this population with elevated tryptase, one patient was allergic to Diptera: this case report had already been published a few years ago [36, 37] but, at this time, tryptase was not measured. To our knowledge, this is the first case of allergy to Diptera with elevated tryptase reported in the literature. Surprisingly, patients with elevated tryptase experienced more recurrent anaphylactic reactions to venom than patients with normal BT, although patients with elevated tryptase were not more exposed to insects. Venom immunotherapy was not delayed after the first systemic reaction in patients with elevated BT, compared with patients with normal tryptase. A decreased efficacy of immunotherapy has been reported in patients with mastocytosis [1, 5, 8, 18] or elevated tryptase [16], but, in the present study, recurrent reactions in patients with elevated tryptase occurred before the initiation of immunotherapy. No clear explanation can be proposed for this observation.

MC mediator release in mastocytosis can induce anaphylaxis, which appears more frequent than in the normal population [29, 38–40]. In the most recent series, the cumulative incidence of anaphylaxis in 120 patients with mastocytosis was 49% in adults and 9% in children [29]. Hymenoptera stings represent one of the major triggering factors, although the frequency of Hymenoptera anaphylaxis in mastocytosis varies in the published data from 5% to 27% [29, 32, 39]. The frequency of Hymenoptera allergy has not been specifically studied in cutaneous mastocytosis but many cases of systemic reactions to Hymenoptera have been reported in patients with UP [1, 2, 6–9, 31]. In this study population, seven patients had mastocytosis: one case of CM with no proven systemic involvement and six cases of ISM, with cutaneous mastocytosis in four cases. In five out of cases, mastocytosis had not been diagnosed previously. 3.1% of the population of 131 venom-allergic patients included with the same criteria presented mastocytosis. The prevalence of mastocytosis is not known but the incidence of cutaneous mastocytosis is seven new cases per 1 000 000 individuals each year [41]. Epidemiologic studies report a prevalence of systemic anaphylactic sting reactions between 0.6% and 3.3% [42]. Although mastocytosis may have been missed in patients with normal or slightly elevated tryptase, these results suggest that mastocytosis is more frequent in patients with venom allergy. These results are fairly similar to previously published data [16–18].

All cases of cutaneous mastocytosis observed in the present study were of UP type, which usually accounts for 90% of CM. Skin lesions mainly involved the trunk, a common site of CM. Rash was not systematically symptomatic: pruritus, reported in 27–71% of series, was reported by two out of five patients [43–47]. However, the cases of cutaneous mastocytosis observed in our study presented several unusual clinical features. Skin lesions were inconspiscuous in most patients. Darier's sign was absent in two out of five patients, although it has been reported to be present in more than 85% of cases of CM in the literature [43–45]. Consequently, a thorough dermatological examination is essential to avoid missing the diagnosis of discrete cutaneous mastocytosis.

Six of the 23 patients with elevated tryptase had bone marrow involvement. While bone biopsy showed a dense multifocal MC infiltrate in all cases, bone marrow biopsy failed to demonstrate this type of infiltrate in five of these cases. Bone marrow biopsy represents the gold standard for the diagnosis of systemic mastocytosis. Although a bone marrow biopsy presents a high specificity for the detection of MC, its sensitivity is limited due to the heterogeneous distribution and the low frequency of MC in the bone marrow [26–48]. A bone biopsy was performed in this study in order to assess bone histomorphometric parameters (paper in preparation) but appeared to be more discriminating for the diagnosis of systemic involvement, no doubt because of the larger bone marrow sample and the more specific sampling method. Systemic mastocytosis could not be confirmed according to consensus criteria in five cases, despite a diffuse MC infiltrate in the bone marrow. Detection of c-kit mutation and flow cytometry to study CD2/CD25 expression on MC are other minor criteria for the diagnosis of systemic mastocytosis but are not performed routinely in our hospital. Consequently, the criteria for SM were not met in these cases. However, these patients were considered to be at risk for systemic mastocytosis: regular clinical and laboratory follow-up will be performed to detect any progression of the disease.

These results may have several consequences for the management of allergic patients: a severe anaphylactic reaction with flushing, but without urticaria, may be an indication for BT assay and screening for mastocytosis. When this type of atypical reaction is observed in the Department of Allergology, a dermatological examination is performed and bone marrow investigations are

proposed. The severity of venom reactions in patients with mastocytosis may justify life-long immunotherapy, although a decreased efficacy in immunotherapy has been frequently observed in these patients. These patients also present an increased risk of repeated anaphylactic reactions. They should therefore be informed about possible causative factors, especially in the case of general anaesthesia, and, in case of anaphylaxis, should be taught emergency medications and techniques for self-administration of epinephrine. In addition, some patients with mastocytosis may develop signs of disease progression related to organ infiltration or associated clonal haematologic non-MC lineage disorder (from $<5\%$ up to 20% in some series) [14]. As recommended by the consensus proposal, regular clinical and laboratory follow-up should be performed in patients with mastocytosis.

Conclusion

In conclusion, these results indicate a particular clinical presentation of the anaphylactic reaction in venom-allergic patients with elevated tryptase, characterized by greater severity, more frequent flushing and less frequent urticaria. They underlined the higher frequency of mastocytosis in this population. As most of these patients with mastocytosis are relatively asymptomatic, systematic screening consisting of a dermatological examination, and a bone marrow biopsy is required for the diagnosis. This study suggests a clinical indicator for the detection of patients at a high risk of mastocytosis: tryptase assay should be performed in every patient with a systemic reaction to venom and mastocytosis considered in patients with a severe anaphylactic reaction with flushing and absence of urticaria. The severity and recurrence of anaphylactic episodes in these patients may justify a life-long immunotherapy and education in emergency medications, in order to prevent fatal events.

References

1 Müller UR, Horat W, Wüthrich B, Conroy M, Reisman RE. Anaphylaxis after Hymenoptera stings in three patients with urticaria pigmentosa. *J Allergy Clin Immunol* 1983; **72**:685–9.

2 Price LA, Safko M. Bee venom allergy in a patient with urticaria pigmentosa. *J Allergy Clin Immunol* 1987; **79**:407–9.

3 Kors JW, Van Doormaal JJ, De Monchy JGR. Anaphylactoid shock following Hymenoptera sting as a presenting symptom of systemic mastocytosis. *J Intern Med* 1993; **233**:255–8.

4 Engler RJ, Davis WS. Rush Hymenoptera venom immunotherapy: successful treatment in a patient with systemic mast cell disease. *J Allergy Clin Immunol* 1994; **94**:556–9.

5 Oude Elberink JN, de Monchy JG, Kors JW, van Doormaal JJ, Dubois AE. Fatal anaphylaxis after a yellow jacket sting, despite venom immunotherapy, in two patients with mastocytosis. *J Allergy Clin Immunol* 1997; **99**:153–4.

6 Fricker M, Helbling A, Schwartz L, Müller U. Hymenoptera sting anaphylaxis and urticaria pigmentosa: clinical findings and results of venom immunotherapy in ten patients. *J Allergy Clin Immunol* 1997; **100**:11–5.

7 Wein M. Sting anaphylaxis and urticaria pigmentosa. *J Allergy Clin Immunol* 1998; **101**:432.

8 Kim DC, Horan R. Anaphylaxis to insect sting associated with urticaria pigmentosa. *Allergy Asthma Proc* 2003; **24**:175–8.

9 Kraemer JP, Hutt N, De Blay F, Pauli G. Severe anaphylactic reactions to hymenoptera stings and cutaneous mastocytosis: interest and difficulties of immunotherapy. *Rev Fr Allergol* 2004; **44**:414–6.

10 Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 1987; **316**:1622–6.

11 Schwartz LB, Bradford TR, Rouse C *et al*. Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. *J Clin Immunol* 1994; **14**:190–204.

12 Schwartz LB, Sakai K, Bradford TR *et al*. The α form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. *J Clin Invest* 1995; **96**:2702–10.

13 Schwartz LB. Clinical utility of tryptase levels in systemic mastocytosis and associated hematologic disorders. *Leuk Res* 2001; **25**:553–62.

14 Valent P, Horny HP, Escribano L *et al*. Diagnostic criteria and classification of mastocytosis: a consensus proposal. *Leuk Res* 2001; **25**:603–25.

15 Valent P, Horny HP, Li CY *et al*. Mastocytosis. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *World health organization classification of tumours. Pathology and genetics. Tumours of haematopoietic and lymphoid tissues, Vol. 1*. Lyon: IARC, 2001; 291–302.

16 Haeberli G, Brönnimann M, Hunziker T, Müller U. Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. *Clin Exp Allergy* 2003; **33**:1216–20.

17 Ludolph-Hauser D, Ruëff F, Fries C, Schöpf P, Przybilla B. Constitutively raised serum concentrations of mast cell tryptase and severe anaphylactic reactions to Hymenoptera stings. *Lancet* 2001; **357**:361–2.

18 Ruëff F, Placzek M, Przybilla B. Mastocytosis and Hymenoptera venom allergy. *Curr Opin Allergy Clin Immunol* 2006; **6**:284–8.

19 Sainte-Laudy J, Sabbah A, Drouet M, Lauret MG, Loiry ML. Diagnosis of venom allergy by flow cytometry. Correlation with clinical history, skin tests, specific IgE, histamine and leukotriene C4 release. *Clin Exp Allergy* 2000; **30**:1166–71.

20 Fraitag-Spinner S. Cutaneous mastocytosis. *Ann Dermatol Venereol* 2007; **134**:589–92.

21 Lortholary O, Audouin J, Le Tourneau A, Diebold J. The mast cell and its pathology. 2: histopathology of mastocytosis. *Ann Pathol* 1991; **11**:92–100.

22 Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin N Am* 2006; **26**:451–63.

23 Dugas-Breit S, Schöpf P, Dugas M, Schiffl H, Ruëff F, Przybilla B. Baseline serum levels of mast cell tryptase are raised in hemodialysis patients and associated with severity of pruritus. *J Dtsch Dermatol Ges* 2005; **3**:343–7.

24 Kanthawatana S, Carias K, Arnaout R, Hu J, Irani AM, Schwartz LB. The potential clinical utility of serum α-protryptase levels. *J Allergy Clin Immunol* 1999; **103**:1092–9.
25 Sperr WR, Jordan JH, Fiegl M *et al*. Serum tryptase levels in patients with mastocytosis: correlation with mast cell burden and implication for defining the category of disease. *Int Arch Allergy Immunol* 2002; **128**:136–4.
26 Proelss J, Wenzel J, Ko Y, Bieber T, Bauer R. Tryptase detection in bone marrow blood: a new diagnostic tool in systemic mastocytosis. *J Am Acad Dermatol* 2007; **56**:453–7.
27 Webb LM, Lieberman P. Anaphylaxis: a review of 601 cases. *Ann Allergy Asthma Immunol* 2006; **97**:39–43.
28 Kemp SF, Lockey RF, Wolf BL, Lieberman P. Anaphylaxis. A review of 266 cases. *Arch Intern Med* 1995; **155**:1749–54.
29 Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. *Allergy* 2008; **63**:226–32.
30 Koterba AP, Akin C. Differences in the clinical presentation of anaphylaxis in patients with indolent systemic mastocytosis (ISM) versus idiopathic anaphylaxis (AI). *J Allergy Clin Immunol* 2008; **121**:S68 (abstract 263).
31 Biedermann T, Ruëff F, Sander CA, Przybilla B. Mastocytosis associated with severe wasp sting anaphylaxis detected by elevated serum mast cell tryptase levels. *Br J Dermatol* 1999; **141**:1110–2.
32 Florian S, Krauth MT, Simonitsch-Klupp I *et al*. Indolent systemic mastocytosis with elevated serum tryptase, absence of skin lesions and recurrent severe anaphylactoid episodes. *Int Arch Allergy Immunol* 2005; **136**:273–80.
33 Sonneck K, Florian S, Müllauer L *et al*. Diagnostic and subdiagnostic accumulation of mast cells in the bone marrow of patients with anaphylaxis: monoclonal mast cell activation syndrome. *Int Arch Allergy Immunol* 2007; **142**:158–64.
34 Sturm GJ, Heinemann A, Schuster C *et al*. Influence of total IgE levels on the severity of sting reactions in Hymenoptera venom allergy. *Allergy* 2007; **62**:884–9.
35 Müller U, Helbling A, Hunziker T *et al*. Mastocytosis and atopy: a study of 33 patients with urticaria pigmentosa. *Allergy* 1990; **45**:597–603.
36 Sabbah A, Sainte-Laudy J, Hassoun S, Drouet M, Lauret MG, Loiry ML. Allergy to mosquitoes and use of new immunobiological parameters. *Allerg Immunol (Paris)* 1997; **29**:126–8.
37 Hassoun S, Drouet M, Sabbah A. Anaphylaxis caused by a mosquito: 2 case reports. *Allerg Immunol (Paris)* 1999; **31**:285–7.
38 Travis WD, Li CY, Bergstralh EJ, Yam LT, Swee RG. Systemic mast cell disease. Analysis of 58 cases and literature review. *Medicine (Baltimore)* 1988; **67**:345–68.
39 Horan RF, Austen KF. Systemic mastocytosis: retrospective review of a decade's clinical experience at the Brigham and Women's Hospital. *J Invest Dermatol* 1991; **96**:5–13S.
40 González de Olano D, de la Hoz Caballer B, Núñez López R *et al*. Prevalence of allergy and anaphylactic symptoms in 210 adult and pediatric patients with mastocytosis in Spain: a study of the Spanish network on mastocytosis (REMA). *Clin Exp Allergy* 2007; **37**:1547–55.
41 Rosbotham JL, Malik NM, Syrris P *et al*. Lack of c-kit mutation in familial urticaria pigmentosa. *Br J Dermatol* 1999; **140**:849–52.
42 Bilo BM, Rüeff F, Mosbech H *et al*. Diagnosis of Hymenoptera venom allergy. *Allergy* 2005; **60**:1339–49.
43 Topar G, Staudacher C, Geisen F *et al*. Urticaria pigmentosa: a clinical, hematopathologic, and serologic study of 30 adults. *Am J Clin Pathol* 1998; **109**:279–85.
44 Tebbe B, Stavropoulos PG, Krasagakis K, Orfanos CE. Cutaneous mastocytosis in adults. Evaluation of 14 patients with respect to systemic disease manifestations. *Dermatology* 1998; **197**:101–8.
45 Akoglu G, Erkin G, Cakir B *et al*. Cutaneous mastocytosis: demographic aspects and clinical features of 55 patients. *J Eur Acad Dermatol Venereol* 2006; **20**:969–73.
46 Czarnetzki BM, Behrendt H. Urticaria pigmentosa: clinical picture and response to oral disodium cromoglycate. *Br J Dermatol* 1981; **105**:563–7.
47 Middelkamp Hup MA, Heide R, Tank B, Mulder PG, Oranje AP. Comparison of mastocytosis with onset in children and adults. *J Eur Acad Dermatol Venereol* 2002; **16**:115–20.
48 Butterfield JH, Li CY. Bone marrow biopsies for the diagnosis of systemic mastocytosis: is one biopsy sufficient? *Am J Clin Pathol* 2004; **121**:264–7.