

The presence of mast cell clonality in patients with unexplained anaphylaxis

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Summary

Background The mechanisms by which mast cells in patients with unexplained anaphylaxis (UEA) are triggered remain elusive. Onset of episodes is unpredictable and often recurrent. The substantial overlap between the clinical manifestations of UEA and clonal mast cell disorders (CMD) suggests an association between these rare disorders. The two forms of CMD characterized to date are systemic mastocytosis (SM) and monoclonal mast cell activation syndrome (MMAS).

Objective To examine the hypothesis that the pathogenesis of UEA reflects the presence of aberrant subpopulations of mast cells.

Methods Thirty (14 men, 16 women) patients (≥ 18 years) suffering from UEA and with no signs of cutaneous mastocytosis were recruited. Patients underwent an initial complete allergy work-up to confirm the diagnosis of UEA. Level of baseline serum tryptase (sBT) and total IgE were determined. In addition, a bone marrow examination was performed on all 30 patients to investigate possible underlying CMD.

Results Fourteen (47%) of our cases (nine men, five women) were diagnosed with CMD: 10 with SM and four with MMAS. Four of the 10 patients with SM had mast cell aggregates in their bone marrow. All patients with SM exhibited a sBT level > 11.4 ng/mL, whereas this level was elevated in only two of those with MMAS and four with UEA but not diagnosed with CMD. Total IgE levels were lower in the group of patients with CMD ($P < 0.03$).

Conclusion and Clinical Relevance The pathogenic mechanism underlying UEA could be explained by the presence of immunophenotypically aberrant mast cells with clonal markers in 47% of our subjects, indicating that clonal mast cell disorders are present in a substantial subset of these patients. Thus, the presence of CMD should be considered in patients with UEA if they have an elevated level of sBT (≥ 11.4 ng/mL) and cardiovascular symptoms such as syncope.

Keywords CD25, clonal mast cell disorders, idiopathic anaphylaxis, IgE, *KIT* D816V mutation, mast cell clonality, mastocytosis, tryptase, unexplained anaphylaxis

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Introduction

Anaphylaxis, an acute, sudden and potentially fatal hypersensitivity reaction involving multiple organ systems, can be triggered by various exogenous factors, including food, venom, and drugs [1]. However, for certain patients with anaphylaxis, no eliciting factor can be identified despite a comprehensive allergy workup, and therefore, the term 'idiopathic anaphylaxis' (IA)

was introduced in the late 1970s [2]. The reported prevalence of 'IA' varies widely from 7% [3] to 59% [4] of patients with anaphylaxis.

The clinical manifestations of 'IA' and IgE-mediated anaphylaxis are the same, that is, various combinations of urticaria, angio-oedema, tachycardia, pruritus, flushing, hypotension, syncope or presyncope, wheezing, stridor, nausea, abdominal cramps, and diarrhoea, with any given patient usually exhibiting the same pattern

of symptoms during different attacks [5]. The onset of episodes of 'IA' is unpredictable and usually recurrent. Although various theories concerning the pathogenesis of 'IA' have been proposed, none adequately explains the underlying mechanism(s) [6, 7]. To date, mast cells and/or basophils have not been shown to exhibit a lower response threshold for release of their mediators in such patients. To complicate things even more, the terms 'IA' and 'unexplained anaphylaxis (UEA)' are often used interchangeably in the current literature. This disorder therefore remains a perplexing challenge for the clinical allergist.

At the same time, the substantial overlap between the signs and symptoms of the recently recognized clonal mast cell disorders (CMD) and 'IA' has motivated a search for an association between these two rare conditions. Interestingly, this notion is supported by the detection of mast cells bearing clonal markers in five of 12 patients with 'IA' [8]. Clonal mast cell disorders comprise a heterogeneous group of diseases, among which there are two well-characterized forms to date, that is, systemic mastocytosis (SM) and monoclonal mast cell activation syndrome (MMAS) [9, 10]. Common to these two conditions is mast cell clonality, as reflected in a mutation in codon 816 of *KIT* and/or the presence of immunophenotypically aberrant mast cells expressing CD2 and/or CD25 [8, 11, 12].

The well-established diagnosis of SM requires the presence of the major criterion (multifocal mast cell (MC) infiltrates in the bone marrow (BM) or other extracutaneous organs) in combination with one minor criterion or, alternatively, the presence of at least three minor criteria [12, 13]. These minor criteria are abnormal morphology of extracutaneous MCs, a *KIT* D816V mutation in an extracutaneous organ, expression of CD2 and/or CD25 on BM MCs, and a consistently high level of serum tryptase (> 20 ng/mL) [13]. The term MMAS has been applied to patients who do not fulfil the criteria for SM, but demonstrate signs of mast cell clonality [8, 11, 12].

Clinical manifestations of CMD are protean, and intensity of symptoms fluctuates over time. In particular, patients often suffer from episodic or chronic symptoms that relate to mast cell mediator release, such as anaphylaxis [8–10, 14–16]. Also it has been suggested that certain patients with 'idiopathic anaphylaxis' may have an underlying mast cell disorders [8, 17, 18]. The present investigation was designed to examine the hypothesis that an as yet undetected hyperreactive population of mast cells may underlie the pathogenesis of UEA. To this end, possible mast cell clonality in the BM of patients with UEA was explored. In addition, we attempted to identify predictive marker(s) that can distinguish between different UEA phenotypes.

Methods

Patients and procedures

In 2006, the Mastocytosis Centre Karolinska was established at Karolinska University Hospital and Karolinska Institutet. By 31 December 2013, a total of 206 consecutive adult patients (≥ 18 years) with clinically suspected CMD were referred to the centre. All patients underwent medical evaluation, including bone marrow biopsy, to determine potential underlying systemic mast cell disease. Diagnoses of systemic mastocytosis or monoclonal mast cell activation syndromes were made using the current WHO criteria [12, 13], employing BM investigation to confirm the presence/absence of major criterion and/or minor criteria.

Study design

Data were collected retrospectively through review of electronic patient records. As illustrated in Fig. 1, of the 206 patients investigated, 95 presented with anaphylaxis. Among these 95 patients, we have further identified 36 patients (≥ 18 years) who were referred to the allergy outpatient clinic at Karolinska University Hospital Huddinge between February 2006 and November 2013 for one or more episodes of unexplained anaphylaxis. After excluding six of these who exhibited urticaria pigmentosa, the remaining 30 (14 men, 16 women) without signs of mastocytosis in the skin were enrolled in this study. Ethical approval was obtained from the Regional Ethical Review Board, Stockholm, Sweden (Approval number: 2011/1750-31/3), and all 30 patients included were informed about the study and thereafter provided their written consent to participate.

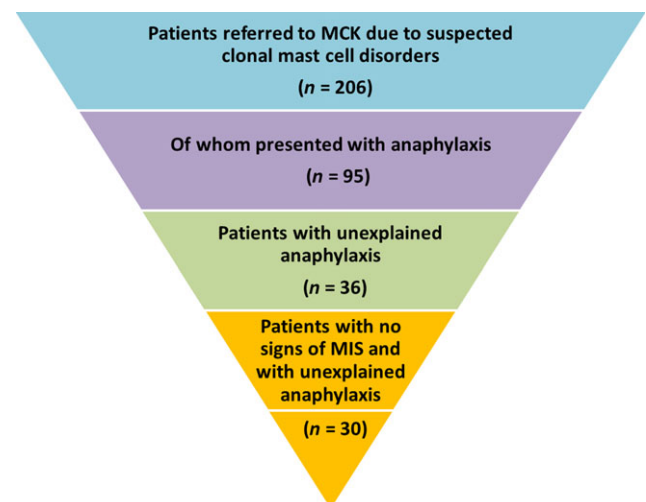


Fig. 1. Source population and selection of the 30 patients with unexplained anaphylaxis involved in this study. MCK, Mastocytosis Center Karolinska; MIS, mastocytosis in the skin.

Before being subjected to bone marrow biopsy in accordance with clinical routines, these 30 patients had initially undergone a thorough clinical and physical examination, along with an allergy work-up (as detailed below), on the basis of which UEA was diagnosed through exclusion of potential elicitors, including foods, drugs, exercise, insect bites or other precipitating factors. Their baseline serum tryptase (sBT) levels (normal reference value < 11.4 ng/mL) were measured (ImmunoCAP, ThermoFisher Scientific, Uppsala, Sweden). Using procedures described previously, the morphology, histology, and immunohistochemistry [19] of the BM MCs were assessed in all 30, and in addition, flow cytometry [20] and analysis of the D816V mutation [21] were performed in 26. In this manner, the absence/presence of mast cell clonality according to current WHO criteria [12, 13] was confirmed. Patients with no bone marrow pathology were considered to suffer from true 'idiopathic anaphylaxis' (IA).

Furthermore, after their initial visits, we evaluated disease activity in these patients prospectively through follow-up visits to the allergy clinic until 31 December 2013.

Allergy work-up

All patients underwent a thorough allergological evaluation, including investigation of their medical histories, carried out by an experienced allergist, and diagnosis involving skin and *in vitro* allergy tests; and, when warranted, provocation tests such as drug or food. All were questioned about the presence of mediator-related symptoms and the onset, frequency, and duration of their episodes. In accordance with the NIAID clinical criteria [22], anaphylactic reactions were diagnosed when either reduced blood pressure or associated symptoms (such as syncope/presyncope), and/or respiratory compromise or laryngeal oedema were present, accompanied by the involvement of the skin-mucosal tissue or gastrointestinal symptoms. For patients whose histories and symptoms were suggestive of hereditary angio-oedema, complement levels were evaluated. We also ruled out conditions such as pheochromocytoma, carcinoma, and psychiatric disorders. When no precipitating factors were detected and other obvious causes eliminated, the exclusion diagnosis of UEA was made.

Skin prick testing (SPT) was performed with commercial extracts (ALK Allergologisk Laboratorium A/S, Horsholm, Denmark) of standard aeroallergens (birch, grass and mugwort pollens; cat, dog, and horse danders; dust mites; moulds) and food allergens (milk, egg, nuts, cereals, fish, shrimp), and hymenoptera venom (bee and wasp). None of the subject had taken antihistamines during the 5 days preceding SPT. The positive control was histamine dihydrochloride (10 mg/mL) and

the negative control 0.9% saline. In accordance with EAACI guidelines [23], a positive skin test was defined as a wheal whose longest dimension was ≥ 3 mm.

Alcohol, food, drugs, exercise, and menstruation were excluded as eliciting factors by ruling out any possible temporal correlation with the anaphylactic reactions. When necessary, the specific IgE antibody test (ImmunoCAP Phadiatop®, ThermoFisher Scientific) was employed as a complementary tool with values > 0.35 kU/L being considered positive. Total serum IgE was measured in all patients.

Statistical analysis

The statistical analyses were performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA), and a *P*-value of < 0.05 was considered to be statistically significant. Median values and ranges are presented to describe continuous variables and frequencies for categorical variables. Because the distribution of these data was not normal, group differences were analysed using the Mann–Whitney *U*-test. Categorical variables were analysed with the Fisher's exact test. Test for correlation was made with Spearman's rank correlation coefficient.

Results

Characteristics of the patients

As presented in Table 1, of our patients, 47% and 53% were men and women, respectively, and their median age was 53 years (range, 25–75 years). Fourteen (47%; nine men and five women) of the 30 were diagnosed with CMD, 10 with SM and four with MMAS. Four of the 10 with SM fulfilled the major criterion, while the

Table 1. Demographic, diagnostic, and laboratory characteristics of patients with unexplained anaphylaxis after BM investigation

Total, <i>n</i> = 30	CMD		
	SM	MMAS	IA
Age ≥ 18 years			
Number (%)	10/30 (33%)	4/30 (14%)	16/30 (53%)
Male gender, <i>n</i> = 14/30 (47%)	7/10 (70%)	2/4 (50%)	5/16 (31%)
MC clusters in the BM	4/10 (40%)	0/4	0/16
CD25 ⁺ MC	10/10	4/4	0/16
<i>KIT</i> mutation D816V	4/6 (67%)	0/4	0/16
	4 n/a		
sBT ≥ 20 ng/mL	8/10 (80%)	1/4 (25%)	0/16
sBT ≥ 11.4 –< 20 ng/mL	2/10 (20%)	1/4 (25%)	4/16 (25%)

CMD, clonal mast cell disorders; SM, systemic mastocytosis; MMAS, monoclonal mast cell activation syndrome; IA, idiopathic anaphylaxis; MC, mast cell; BM, bone marrow; sBT, serum baseline tryptase; n/a, not analysed.

remaining six exhibited at least three minor criteria. All patients with SM in this study had indolent variant of the disease.

Among the six of the 10 patients with SM analysed for the D816V mutation, four carried the *KIT* D816V mutation, which was not detected in any of the remaining 20 patients. Evaluation of the aberrant phenotype markers CD2/CD25 on BM MCs in all patients by immunohistochemical staining and in 26 by flow cytometry as well revealed that all 14 with CMD expressed CD25 and eight of these 14 expressed CD2 as well. None of the remaining 16 patients exhibited signs of mast cell clonality and they were thus classified as IA. The overall median sBT level was 14 ng/mL (range, 1–160).

Atopic sensitization, that is, sensitization to aeroallergens, was observed in 13 (43%) of the 30 patients with UEA, most of whom exhibited sensitization to more than one allergen. The median level of total IgE was 46.5 kU/L (range, 4.4–1600). The most common allergens to which sensitization was detected by the SPT were pollens in 43% (13/30), followed by animal dander in 13% (4/30) and dust mites in 10% (3/30). However, not all of the sensitized patients demonstrated clinical symptoms of allergic disease. For this reason, these test results were scrutinized carefully, and we identified atopic diseases in nine of the 30 patients (30%), four with rhinoconjunctivitis, three with asthma, and two with both asthma and rhinoconjunctivitis.

Clinical presentation

The most frequent mediator-related symptoms were associated with the cardiovascular system (e.g. syncope, hypotension). Syncope was observed in 93% (28/30) of the patients. This was followed by gastrointestinal

symptoms (including abdominal cramps, diarrhoea, and nausea) in 67% (20/30). Less common were cutaneous symptoms such as flushing in 57% (17/30), pruritus in 27% (8/30), and urticaria and angio-oedema in 50% (15/30). Respiratory symptoms (such as wheezing, dyspnoea, and cough) were exhibited by 40% (12/30). The patients often presented with preceding cutaneous symptoms such as flushing or urticaria with/without angio-oedema (which never occurred without urticaria), as syncope was never the only symptom. Thus, in accordance with previous reports [24–26], of the all patients were classified as ‘idiopathic anaphylaxis-generalized’ upon presentation. Late-phase reactions (2 h after two original anaphylactic episodes) were observed in one patient with SM only and required intensive care.

Comparison of patients with CMD and IA

On the basis of retrospective review of the data, the patients were divided arbitrarily into two groups according to their levels of sBT and number of anaphylactic episodes experienced prior to bone marrow examination. Of the 16 (53%) patients with elevated sBT levels (≥ 11.4 ng/mL), 12 patients (75%) obtained a final diagnosis of CMD (Table 2). In contrast, of the 14 patients with normal sBT levels (< 11.4 ng/mL), who were examined after the occurrence of at least three episodes of UEA, only two (14%) were diagnosed with CMD. We also applied an existing prescreening tool, the REMA score [27] of the Spanish Network on Mastocytosis, retrospectively to our subjects (Table 2). REMA score [27] is the sum of positive and negative points as follows: male (+1), female (–1), sBT < 15 ng/mL (–1) or > 25 ng/mL (+2), presence (–2) or absence (+1) of

Table 2. Referral-based characteristic of patients with UEA and retrospective comparison of different predictivity tools for determining underlying CMD

Predictivity indicators	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Patients with normal sBT (< 11.4 ng/mL) and with ≥ 3 UEA episodes	14% (2.2%–42.8%)	25% (7.4%–52.4%)	14% (2.2%–42.8%)	25% (7.4%–52.4%)
Patients with elevated sBT (≥ 11.4 ng/mL) and with ≥ 1 UEA episodes	86% (57.2%–97.8%)	75% (47.6%–92.6%)	75% (47.6%–92.6%)	86% (57.2%–97.8%)
*REMA score (≥ 2) with a sBT cut-off 15–25 ng/mL	86% (57.2%–97.8%)	94% (69.7%–99.0%)	92% (63.9%–98.7%)	88% (63.5%–98.2%)
†Modified REMA score (≥ 2) with a sBT cut-off 11.4–20 ng/mL	93% (66.1%–98.8%)	94% (69.7%–99.0%)	93% (66.1%–98.8%)	94% (69.7%–99.0%)

UEA, unexplained anaphylaxis; CMD, clonal mast cell disorders; CI, confidence interval; sBT, serum baseline tryptase.

*REMA score [27] is the sum of positive and negative points as follows: male (+1), female (–1), sBT < 15 ng/mL (–1) or > 25 ng/mL (+2), presence (–2) or absence (+1) of pruritus, hives or angio-oedema, and presence (+3) of presyncope or syncope. A score of ≥ 2 is considered to be positive and indicates a high probability for CMD, thereby warranting a bone marrow examination.

†Modified REMA score consisted of the sum of positive and negative points as follows: male (+1), female (–1), sBT < 11.4 ng/mL (–1) or > 20 ng/mL (+2), presence (–2) or absence (+1) of pruritus, hives or angio-oedema, and presence (+3) of presyncope or syncope.

pruritus, hives or angio-oedema, and presence (+3) of presyncope or syncope; and a score of ≥ 2 indicates a high probability for CMD. In overall, 13 (43%) patients had a positive REMA score (≥ 2), of whom 12 (92%) obtained the final diagnoses of CMD.

Among the clinical manifestations (Fig. 2), syncope was most common in both groups. However, urticaria/angio-oedema occurred significantly more often in the patients with IA ($P = 0.001$), whereas flushing was more prevalent in the CMD group ($P < 0.04$). There were no significant differences between these groups with respect to gastrointestinal and respiratory symptoms.

As expected, sBT levels were significantly higher in patients with CMD than in those with IA ($P < 0.001$). Table 3 summarizes additional characteristics of these two groups. Total IgE levels were significantly higher in the IA group ($P < 0.03$), resulting in an inverse correlation between these levels and those of sBT (Fig. 3). Otherwise, there were no significant differences between the groups with respect to age, sex, presence/absence of atopic sensitization and atopic disease, or number of anaphylactic episodes.

Clinical course

As demonstrated in Table 4, the subjects included in the study had experienced a total of 148 unexplained anaphylactic episodes, 119 of which occurred prior to allergy work-up. Three of the 30 patients visited our clinic after a single episode only and four after two episodes; and all of these seven patients demonstrated elevated sBT (median 23 ng/mL; range, 15–64). The remaining 23 patients had experienced at least three UEA episodes before being referred to us. Only one experienced frequent episodes, according to the original

classification [24, 25], that is, $> 5/\text{year}$, whereas the others reported episodes scattered over several years.

During the follow-up, seven of the 30 subjects had experienced a total of 29 new UEA episodes (Table 4). After allergy work-up, all patients were advised to self-administer intramuscular adrenaline upon commencement of acute symptoms and instructed to go to the emergency ward for objective assessment; however, only 71% (5/7) patients used adrenaline, and 2 (29%) of the seven also sought emergency care. All also received H1-antihistamines and oral steroids during acute episodes. Interestingly, one of these seven patients accounted for 16 of 29 follow-up episodes (Table 4). This patient used adrenaline in all episodes and afterwards sought emergency ward in most of the episodes.

For maintenance therapy, 25 of the patients received mediator blockers, such as antihistamines (mainly H1-blockers), on a regular base. Seven also received antileukotrienes and four of these seven received triple combination therapy with H1- and H2-histamine blockers and antileukotrienes (Table 4). Doses were adjusted on the basis of the severity of previous reactions, as well as the frequency of episodes. None of our patients took oral steroids on a regular basis.

Three of our cases were treated with the anti-IgE, omalizumab, starting with the standard dose [28] of 300 mg, given twice monthly. The first of these patients, who suffered from MMAS with a total of eight UEA episodes, gained no benefit despite 6 months of trial. The indication for omalizumab therapy in the other two (both with SM and severe episodes of UEA) was the severe nature of their reactions, which seriously lowered their quality of life. They experienced no new episodes during 32 months of omalizumab administration (at the same dose, but administered once monthly

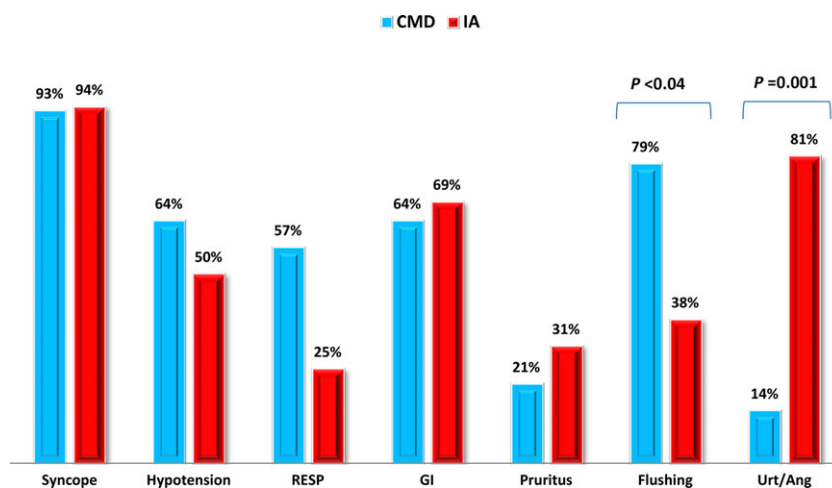


Fig. 2. Distribution of clinical manifestations associated with the most severe episodes of unexplained anaphylaxis in patients with CMD ($n = 14$) vs. IA ($n = 16$). IA, idiopathic anaphylaxis; CMD, clonal mast cell disorders; RESP, respiratory symptoms; GI, gastrointestinal symptoms; Urt/Ang, urticaria and/or angio-oedema.

Table 3. Clinical and demographic characteristics of the patients with CMD vs. IA

Total, <i>n</i> = 30	CMD (<i>n</i> =14)	IA (<i>n</i> =16)	<i>P</i>
Age ≥ 18 years			
Age, years			
Median	57	44	NS
(Range)	(25–75)	(25–69)	
Male gender	9/14 (64%)	5/16 (31%)	NS
Atopic sensitization	6/14 (43%)	7/16 (44%)	NS
Atopic disease (asthma and/or rhinoconjunctivitis)	5/14 (36%)	4/16 (25%)	NS
Total IgE (kE/L)			
Median	25	102	< 0.03
(Range)	(4.4–1600)	(22–1100)	
sBT (ng/mL)			
Median	22.5	6.1	< 0.001
(Range)	(5–160)	(1–17)	
Total number of anaphylactic episodes in all patients	58	90	NS

CMD, clonal mast cell disorders; IA, idiopathic anaphylaxis; NS, not significant; sBT, serum baseline tryptase.

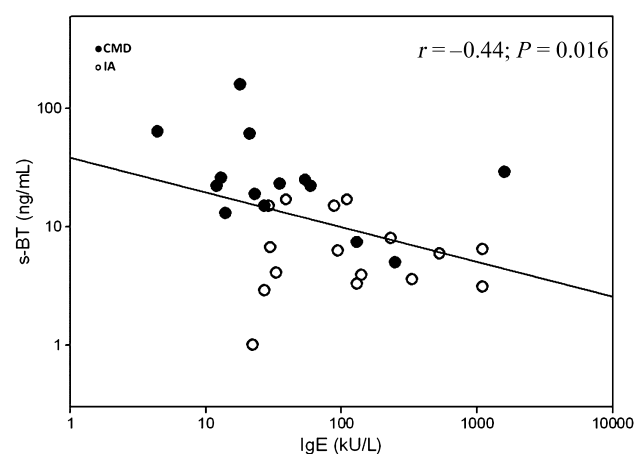


Fig. 3. The inverse correlation between the total serum IgE and baseline serum tryptase levels in all of our 30 subjects. Note that the scales on the X- and Y-axes are logarithmic. *r*, correlation coefficient; sBT, serum baseline tryptase; IgE, immunoglobulin E; IA, idiopathic anaphylaxis; CMD, clonal mast cell disorders.

after 12 months and once every 6 weeks after 24 months) and tolerated this treatment well.

The total period of follow-up at the time of our data analysis (in late December of 2013) was 92.3 patient-years (median 31 months; range, 2–94). At the time of last follow-up, 21 (70%) of the 30 patients with UEA were in remission, that is, had experienced no new anaphylactic episodes for at least 1 year. Among the remaining 9; the frequency of episodes was lowered, that is, improved, for 5 and remained unchanged for 4 (Table 4). None experienced more severe disease, and to

date, no fatalities have occurred. We also made comparison between outcome groups regarding clinical course of the reactions and found that the rate of remission was significantly higher in the patients with CMD than for those with IA ($P < 0.02$).

Discussion

This study documenting the experience of 30 patients referred consecutively to our clinic for one or more episodes of unexplained anaphylaxis represents one of the most comprehensive data to date in the field of idiopathic anaphylaxis. In 47% of these subjects, their episodes of UEA can be explained by the presence of an aberrant mast cell population expressing clonal markers that may contribute to a hyperreactive mast cell phenotype and thereby lower the response threshold.

To determine the presence/absence of an underlying clonal mast cell disorder and thereby provide patients with UEA a definitive diagnosis, a bone marrow examination should be considered. However, the criteria for eligibility for such an examination remain a matter of debate, as no recommendations have yet been established. Here, we proposed that either an elevated level of sBT (≥ 11.4 ng/mL) or, alternatively, three or more episodes of UEA in individual patients with normal sBT levels can predict an as-yet-unrevealed mast cell clonality. Nevertheless, the occurrence of at least three episodes of UEA did not in itself appear to be a reliable prescreening marker, as only two of the 14 patients (14%) with numerous episodes and normal sBT levels were diagnosed with CMD (Table 2). Accordingly, an elevated level of sBT appears to be a more reliable prescreening marker for CMD, demonstrating a sensitivity of 86% and specificity of 75% in our patients.

To compare eligibility criteria, we applied an existing prescreening tool, the REMA score [27], retrospectively to our subjects and found this to be of value in comparison with the finding from the bone marrow investigations. The REMA score exhibited greater specificity than, but a sensitivity similar to that of the sBT level. Application of the REMA score with modified cut-off values for sBT (11.4–20 ng/mL instead of 15–25 ng/mL) resulted in both the best sensitivity (93%) and specificity (94%) (Table 2). While the modified REMA score thus appears to provide a valuable indicator of eligibility for bone marrow examination, an alternative approach would be to analyse the *KIT* D816V mutation in the peripheral blood mononuclear cells of patients [29]. This assay is, however, not yet established in our centre and remains to be evaluated prospectively in UEA.

The 43% overall rate of atopy among our subjects is consistent with previous reports of 43% [30] and 48% [26]. Nonetheless, it is difficult to assess whether UEA develops predominantly in atopic subjects or not, as the

Table 4. Clinical course of individual patients with unexplained anaphylaxis, final diagnosis after bone marrow examination, and follow-up months as of 31 December 2013

Subject ID, Gender	Time of first visit	Number of episodes prior to evaluation	Final diagnosis	Maintenance therapy	Number of episodes at follow-up	Clinical course	Follow-up, months
Nr 1, M	2008/05	5	ISM	H1, H2-blockers Antileukotriene	0	R	67
Nr 2, M	2012/02	3	ISM	H1-blockers	0	R	22
Nr 3, M	2011/04	5	ISM	H1, H2-blockers Antileukotriene Omalizumab	0	R	32
Nr 4, M	2012/03	12	ISM	H1-blockers	0	R	21
Nr 5, M	2011/04	4	ISM	H1, H2-blockers Antileukotriene Omalizumab	0	R	32
Nr 6, M	2009/10	2	ISM	H1, H2-blockers	0	R	52
Nr 7, F	2011/10	4	ISM	H1, H2-blockers Antileukotriene	0	R	26
Nr 8, M	2010/06	2	ISM	H1-blockers	0	R	42
Nr 9, F	2012/07	4	ISM	H1-blockers Antileukotriene	0	R	17
Nr 10, F	2012/11	2	ISM	H1-blockers	0	R	13
Nr 11, M	2007/11	4	MMAS	H1-blockers Omalizumab	4	U	73
Nr 12, F	2011/11	3	MMAS	H1-blockers	0	R	25
Nr 13, M	2012/01	1	MMAS	None	0	R	23
Nr 14, F	2010/03	3	MMAS	H1-blockers	0	R	45
Nr 15, F	2008/04	4	IA	H1-blockers	5	U	68
Nr 16, F	2007/08	4	IA	H1-blockers	16	*I	76
Nr 17, F	2011/03	3	IA	None	1	I	33
Nr 18, F	2011/04	3	IA	H1-blockers	1	I	32
Nr 19, M	2006/02	3	IA	H1-blockers Antileukotriene	0	R	94
Nr 20, F	2006/04	3	IA	None	1	I	92
Nr 21, F	2009/09	3	IA	H1-blockers	0	R	51
Nr 22, M	2010/05	3	IA	H1-blockers	0	R	31
Nr 23, M	2011/08	3	IA	H1-blockers	0	R	28
Nr 24, M	2010/05	5	IA	None	0	R	31
Nr 25, F	2010/10	5	IA	H1-blockers	0	R	26
Nr 26, F	2012/05	19	IA	H1-blockers	1	I	19
Nr 27, F	2012/08	3	IA	H1-blockers	0	R	16
Nr 28, F	2012/11	1	IA	None	0	R	13
Nr 29, F	2013/07	2	IA	H1-blockers	0	[†] U	6
Nr 30, M	2013/11	1	IA	H1-blockers Antileukotriene	0	[†] U	2

ISM, indolent systemic mastocytosis; MMAS, monoclonal mast cell activation syndrome; IA, idiopathic anaphylaxis; M, male; F, female; R, remission (defined as no new reactions during at least last 12 months); I, improved; U, unchanged.

*Considered as 'improved' due to no new reaction during last 12 months.

[†]Considered as 'unchanged' due to short follow-up period (6 vs. 2 months).

prevalence of atopy among adults in the general Swedish population varies from 27% [31] to 39% [32]. However, here, the groups with CMD or IA did not differ with respect to the prevalence of atopy or atopic diseases. Remarkably, total IgE levels were higher in the patients with IA, whereas sBT levels were higher in the CMD group, resulting in an apparent inverse correlation between these two parameters ($P < 0.02$), in line with a previous investigation [8]. Moreover, the patients with unexplained anaphylaxis and CMD were predominantly men and relatively older, although these trends were not statistically significant.

Interestingly, the most frequent episodic manifestations of UEA observed here were syncope (93%) and gastrointestinal symptoms (67%), the incidence of

which differed from previous reports on hypotension or syncope (23% [26] and 9% [33]) and gastrointestinal symptoms (22% [26] and 32% [33]). In addition, urticaria and/or angio-oedema were previously observed to occur in 100% [26] and 91% [33] of such patients, whereas the corresponding value here was only 50%. Notably, none of our patients had experienced angio-oedema without urticaria during an anaphylactic episode. It is also noteworthy that episodes of syncope were preceded by urticaria with/without angio-oedema in our patients with IA, but by flushing in those with CMD. Although the reasons for such discrepancies between different studies are not clear, one possible explanation is the lack of a consensus definition of an anaphylactic reaction and the use of various criteria for

diagnosis. Here, we applied the NIAID clinical criteria [22], whereas the older studies were performed before consensus definitions were available. Heterogeneity in the patient cohorts examined might also have contributed to the discrepancies described above.

From the time of their first visit, our subjects were advised to take adrenaline upon the debut of symptoms and it is impossible to know whether certain symptoms would otherwise have developed into full-blown anaphylaxis. However, because of the potentially life-threatening nature of their reactions [34, 35], we consider it crucial to advise patients to use such emergency medication. The self-reported rate of adrenaline usage was 71% in seven patients with follow-up episodes in this study. With respect to maintenance therapy, preventive usage of oral corticosteroid was not employed as only one patient fulfilled the recommendation for such treatment (> 5 episodes/year) [26] and this patient refused to use long-term steroid prophylaxis. Nonetheless, this same patient achieved spontaneous remission during the last 2 years of the study after having experienced a total of 20 UEA episodes during the preceding 5 years.

Interestingly, the number of patients with resolved disease was significantly higher in the CMD group ($P < 0.02$), perhaps because of their more extensive usage of mediator blockers and/or the larger proportion of men in this group. Certain of the patients with IA discontinued the usage of mediator blockers, as this was not beneficial for them. Although spontaneous recovery of patients has been reported previously [30], we suggest that maintenance therapy with mediator blockers was beneficial for our patients.

A number of reports describe the usage of omalizumab as maintenance therapy for patients with mastocytosis and UEA [28, 36, 37], but underlying mechanism(s) of action remains elusive. Of our three patients treated with omalizumab, the one suffering from MMAS with recurrent, unexplained anaphylactic episodes experienced no beneficial effects, despite 6 months of treatment. However, the remaining two with SM did not experience any new episodes during this therapy. As both had earlier experienced episodes only infrequently (i.e. once every 2–3 years), it is difficult to assess whether their remission was spontaneous or due to omalizumab treatment and/or their regular usage of mediator blockers. We recommend, therefore, that omalizumab treatment should be reserved for patients with severe and frequent (at least 3/year)

episodes who do not respond to other treatment. In this connection, controlled studies are clearly warranted.

Although the diagnosis of UEA was highly reliable here, the findings are from a single mastocytosis centre and may therefore not be generalizable to all patients with UEA. Another concern is potential recall bias, at least to a certain extent, as a number of the UEA episodes were based on patient-reported data and not documented by a physician. The relative paucity of the study subjects investigated is an additional limitation highlighting the pressing need for further research in this area. On the other hand, it is difficult to design such comprehensive studies, as not all patients agree to undergo a procedure as invasive as bone marrow biopsy.

In conclusion, unexplained anaphylaxis appears to be a heterogeneous entity and the situation is made even more complicated by the interchangeable use of the terms 'IA' and 'UEA' in current literature. This is partly because the term 'IA' was coined before the CMD nomenclature had been introduced into practice. A definitive diagnosis of true IA requires careful elimination of all potential triggers of anaphylaxis, as well as other conditions with similar symptoms. Until the presence/absence of CMD is established, using the term unexplained anaphylaxis (UEA) instead of 'IA' is therefore more appropriate.

Our findings are in agreement with a previous report [8] and lend further support to the hypothesis that clonal mast cell disorders present in a substantial subset of patients with UEA, particularly in men who experience cardiovascular symptoms such as syncope and exhibit an elevated level of sBT (≥ 11.4 ng/mL).

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Conflict of interest

TG has received payment for lectures from Novartis and ALK-Abelló. The other authors have no relevant conflict of interests to declare.

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