

Validation of the REMA Score for Predicting Mast Cell Clonality and Systemic Mastocytosis in Patients with Systemic Mast Cell Activation Symptoms

I. Alvarez-Twose^{a, d} D. González-de-Olano^{b, d} L. Sánchez-Muñoz^{a, d} A. Matito^{a, d}
M. Jara-Acevedo^{c, d} C. Teodosio^{c, d} A. García-Montero^{c, d} J.M. Morgado^{a, d}
A. Orfao^{c, d} L. Escribano^{a, d}

^aInstituto de Estudios de Mastocitosis de Castilla La Mancha (CLMast), Hospital Virgen del Valle, Toledo,

^bServicio de Alergología, Hospital de Fuenlabrada, Madrid, ^cServicio General de Citometría, Centro de Investigación del Cáncer/IBMCC (USAL/CSIC) y Departamento de Medicina, Universidad de Salamanca, Salamanca, y ^dRed Española de Mastocitosis (REMA), España

Key Words

Mast cell • Mastocytosis • Mast cell activation syndrome • Tryptase • Clonality

Abstract

Background: A variable percentage of patients with systemic mast cell (MC) activation symptoms meet criteria for systemic mastocytosis (SM). We prospectively evaluated the clinical utility of the REMA score versus serum baseline tryptase (sBt) levels for predicting MC clonality and SM in 158 patients with systemic MC activation symptoms in the absence of mastocytosis in the skin (MIS). **Methods:** World Health Organization criteria for SM were applied in all cases. MC clonality was defined as the presence of *KIT*-mutated MC or by a clonal HUMARA test. The REMA score consisted of the assignment of positive or negative points as follows: male (+1), female (–1), sBt <15 µg/l (–1) or >25 µg/l (+2), presence (–2) or absence (+1) of pruritus, hives or angioedema and presence (+3) of presyncope or syncope. Efficiency of the REMA score for predicting MC clonality and SM was assessed by receiver operating characteristic (ROC) curve analyses and compared to those obtained by means of sBt levels alone. **Results:** Molecular studies revealed the presence of clonal MC in 68/80 SM cases and in 11/78 patients who did not meet the criteria for SM. ROC curve analyses

confirmed the greater sensitivity and a similar specificity of the REMA score versus sBt levels (84 vs. 59% and 74 vs. 70% for MC clonality and 87 vs. 62% and 73 vs. 71% for SM, respectively). **Conclusions:** Our results confirm the clinical utility of the REMA score to predict MC clonality and SM in patients suffering from systemic MC activation symptoms without MIS.

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Introduction

Mast cell (MC) activation disorders (MCAD) are a highly heterogeneous group of diseases characterized by the presence of episodic symptoms attributable to MC mediator release. Recently, a classification of diseases has been proposed associated with MC activation which comprises secondary, idiopathic and primary MC disorders [1]. The primary MC disorders include not only systemic mastocytosis (SM) with or without mastocytosis in the skin (MIS), but also disorders characterized by the presence of clonal MC not fulfilling criteria for SM, which have been the so-called monoclonal MC activation syndrome or clonal MCAD (c-MCAD) [1–3]. Efficient criteria to select for those MCAD patients without MIS with a high probability of having clonal bone marrow (BM)

Table 1. Clinical and serum criteria used by the REMA score (range: -4 to +7) recently proposed to predict BM MC clonality and SM in patients who present with systemic MC mediator release symptoms in the absence of MIS

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
Tryptase ¹	
<15 ng/ml	-1
>25 ng/ml	+2

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MC (usually associated with an underlying SM) for a complete BM study are warranted.

Currently, it is widely accepted that increased serum baseline tryptase (sBt) levels over 20 µg/l are highly suggestive of SM [2, 4]; nevertheless, recent data suggest that a lower threshold of 11.4 µg/l could be more efficient [5]. However, increased sBt levels are not specific of SM and can also be found in other allergic and nonallergic diseases [2, 6, 7]; in turn, normal sBt levels can also be detected in some patients with SM particularly at the early stages of the disease [3]. Recently, the Spanish Network on Mastocytosis (REMA) has built a simple clinical score associated with both a high sensitivity and specificity to predict BM MC clonality [3]. Here we prospectively evaluated the clinical utility of the REMA score versus sBt levels in predicting both MC clonality and the presence of an underlying SM, in an unselected group of 158 patients presenting with systemic and severe MC activation symptoms in the absence of MIS, independently of the *KIT* mutational status and immunophenotypical characteristics of BM MC.

Patients and Methods

Clinical and Allergic Workup

A total of 158 adult patients – 84 (53%) males and 74 (47%) females with a median age of 50 years (range: 17–81) – suffering from severe systemic MC activation-related symptoms in the absence of MIS, who were consecutively referred to the REMA (between February 2001 and October 2010) for screening of SM, were included in the study after informed consent was given by each subject. The clinical signs and symptoms occurring during acute

episodes and their specific triggers were recorded in each case. In patients who suffered from recurrent episodes, only those symptoms involving the most severe one were considered. Anaphylaxis was defined following previous published criteria [8]. Allergic workup included systematic measurement of sBt (CAP, Phadia Diagnostics, Uppsala, Sweden) and total serum IgE. Additional studies including specific IgE (Immuno CAP, Phadia Diagnostics) and skin tests were performed in those cases in which a specific trigger potentially causing an IgE-mediated reaction (e.g. insect sting, drugs or food) was identified.

BM Studies

In every case, a complete BM study was performed strictly following recently proposed criteria [2, 9, 10] including immunophenotypical analysis of BM MC [11, 12], detection of somatic activating exon 17 *KIT* mutations [13] and the pattern of inactivation of chromosome X in FACS-purified BM MC from 6 female patients lacking *KIT* mutations by the HUMARA assay [14].

Classification of Patients according to BM Findings

Following the currently accepted recommendations [2, 4, 10], patients were diagnosed with SM or clonal sMCAD (c-sMCAD) or nonclonal sMCAD (nc-sMCAD) depending on the presence versus absence of *KIT*-mutated MC or a clonal HUMARA test, respectively. In those cases in which molecular studies were either not performed or not valuable, *KIT* mutation was considered as unknown.

REMA Score

The REMA score [3] was used to classify the patients into two groups according to characteristics detailed in table 1. A cutoff value of ≥ 2 was considered to be associated with a high probability of c-sMCAD versus nc-sMCAD. The overall sensitivity and specificity of the REMA score was compared to those obtained by means of sBt levels alone with cutoff values for this latter parameter of 23.1 µg/l.

Statistical Analyses

For all continuous variables, median and range were calculated, while for categorical variables, frequencies were reported. The Mann-Whitney U and the χ^2 tests were used to assess the statistical significance of differences observed between groups for continuous and categorical variables, respectively. The optimal cutoff values of the REMA score and sBt levels for predicting SM and BM MC clonality were calculated by receiver operating characteristic (ROC) curves. Statistical comparisons between ROC curves were performed according to the nonparametric method described by DeLong et al. [15]. *p* values ≤ 0.05 were considered to be associated with statistical significance. All statistical analyses were performed with the SPSS 15.0 statistical software package (SPSS, Chicago, Ill., USA) and the Medcalc software (version 11.4; Medcalc, Mariakerke, Belgium).

Results

Patient Subgroups

Eighty out of 158 patients (51%) were diagnosed with SM. In this group, clonal BM MC were detected in 68/80 cases (85%) while in only 1 case *KIT* mutation was negative; in the remaining 11 patients, molecular studies were

Table 2. MC mediator release symptoms observed throughout the most severe episode in patients with systemic MC activation symptoms (n = 158) distributed according to the final diagnosis established after analysis of the BM

Clinical symptoms	No criteria for SM				SM cases (n = 80)	p value
	unknown <i>KIT</i> mutation (n = 15)	nc-sMCAD (n = 52)	c-sMCAD (n = 11)	non-SM cases (n = 78)		
Skin/mucosa						
Urticaria or pruritus	12 (80)	44 (84.6)	6 (54.5)	62 (79.5)	26 (32.5)	<0.001
Angioedema	7 (46.7)	30 (57.7)	3 (27.3)	40 (51.3)	7 (8.8)	<0.001
Cardiovascular						
Flushing	2 (13.3)	9 (17.3)	3 (27.3)	14 (17.9)	30 (37.5)	0.01
Palpitations	1 (6.7)	12 (23.1)	3 (27.3)	16 (20.5)	17 (21.3)	NS
Dizziness	5 (33.3)	24 (46.2)	4 (36.4)	33 (42.3)	64 (80)	<0.001
Syncope	5 (33.3)	14 (26.9)	6 (54.5)	25 (32.1)	58 (72.5)	<0.001
Hypotension	3 (20)	13 (25)	4 (36.4)	20 (25.6)	37 (46.3)	0.01
Hypertension	1 (6.7)	3 (5.8)	0	4 (5.1)	0	NS
Cardiac arrest	1 (6.7)	2 (3.8)	2 (18.2)	5 (6.4)	5 (6.3)	NS
Respiratory						
Dyspnea	9 (60)	25 (48.1)	7 (63.6)	41 (52.6)	33 (41.3)	NS
Gastrointestinal						
Abdominal cramping	3 (20)	9 (17.3)	2 (18.2)	14 (17.9)	11 (13.8)	NS
Diarrhea	3 (20)	6 (11.5)	0	9 (11.5)	22 (27.5)	0.007
Nausea or vomiting	5 (33.3)	14 (26.9)	3 (27.3)	22 (28.2)	31 (38.8)	NS
Other symptoms						
Headache	2 (13.3)	1 (1.9)	0	3 (3.8)	6 (7.5)	NS
Fever	0	1 (1.9)	0	1 (1.3)	2 (2.5)	NS
Rhinoconjunctivitis	1 (6.7)	4 (7.7)	0	5 (6.4)	7 (8.8)	NS
Paresthesias	1 (6.7)	6 (11.5)	2 (18.2)	9 (11.5)	11 (13.8)	NS

Results are expressed as total number of patients with percentages in parentheses.

p values refer to comparisons between SM cases versus non-SM cases. NS = Statistically not significantly different.

either not performed (n = 5) or not valuable (n = 6) but other WHO criteria for SM were fulfilled.

Among the 78 patients who did not meet criteria for SM, 11 cases (14%) showed *KIT*-mutated BM MC and they were thus classified as c-sMCAD; of note, in 4 of these 11 cases, simultaneous expression of CD25 by BM MC was also found. Fifty-two cases of these 78 patients (67%) showed no clonal/mutated BM MC and they were classified as nc-sMCAD. Finally, the other 15 non-SM patients (19%) were unclassifiable because *KIT* mutational analyses were not performed or they were not valuable due to the low number of BM MC. Among these latter 15 patients, none fulfilled the major criterion for SM and they all had <2 minor criteria (0 or 1).

Clinical Findings

The main clinical symptoms during acute episodes according to the different subgroups are shown in table 2. Most patients suffered from a combination of

symptoms, usually in a stepwise fashion, and they fulfilled criteria for anaphylaxis in 140/158 cases (89%). According to clinical data included in the REMA score, male gender, the presence of dizziness and/or syncope and the absence of hives, pruritus or angioedema were significantly ($p \leq 0.001$) more frequent in SM versus non-SM patients; by contrast, female gender as well as the presence of hives, pruritus or angioedema were significantly ($p \leq 0.001$) more frequent in non-SM cases versus SM patients. Table 3 shows the distribution of different triggers for MC mediator release episodes observed among the distinct subgroups of patients. Overall, the most common trigger was insect (mainly hymenoptera) sting. In 56 cases (35%), no identifiable trigger was found, while in 33 patients (21%) with ≥ 2 episodes, each episode was caused by a different trigger. Insects were also the most common trigger among SM patients (59 vs. 26% in non-SM cases; $p < 0.001$), whereas drug-induced MC mediator release was significantly more frequent

Table 3. Distribution of triggers for episodes of systemic MC-released mediator-associated symptoms in patients with systemic MC activation symptoms (n = 158) classified according to the final diagnosis established after analysis of the BM

Triggers	No criteria for SMS				SM (n = 80)	p value
	unknown <i>KIT</i> mutation (n = 15)	nc-sMCAD (n = 52)	c-sMCAD (n = 11)	non-SM cases (n = 78)		
Insects						
Hymenoptera	3 (20)	11 (21.2)	5 (45.5)	19 (24.4)	40 (50)	
Others/unknown	1 (6.7)	0	0	1 (1.3)	5 (6.3)	
Mixed	0	0	0	0	2 (2.5)	
Total of cases	4 (26.7)	11 (21.2)	5 (45.5)	20 (25.6)	47 (58.8)	<0.001
Drugs						
Antibiotics	2 (13.3)	7 (13.5)	3 (27.3)	12 (15.4)	6 (7.5)	
NSAIDs	2 (13.3)	3 (5.8)	0	5 (6.4)	5 (6.3)	
Other drugs	2 (13.3)	1 (1.9)	1 (9.1)	4 (5.1)	0	
Mixed	0	5 (9.6)	0	5 (6.4)	0	
Total of cases	6 (40)	16 (30.8)	4 (36.4)	26 (33.3)	11 (13.8)	0.004
Food						
Fruits	0	2 (3.8)	0	2 (2.6)	2 (2.5)	
Nuts	1 (6.7)	0	0	1 (1.3)	1 (1.3)	
Fish/seafood	1 (6.7)	7 (13.5)	1 (9.1)	9 (11.5)	7 (8.8)	
Other foods	1 (6.7)	0	1 (9.1)	2 (2.6)	1 (1.3)	
Mixed	0	1 (1.9)	0	1 (1.3)	1 (1.3)	
Total of cases	3 (20)	10 (19.2)	2 (18.2)	15 (19.2)	12 (15)	NS
Idiopathic	9 (60)	22 (42.3)	2 (18.2)	33 (42.3)	23 (28.8)	NS
Other triggers	0	1 (1.9)	1 (9.1)	2 (2.6)	3 (3.8)	NS

Results are expressed as total number of patients with percentages in parentheses.

p values refer to comparisons between SM cases versus non-SM cases. NS = Statistically not significantly different.

among non-SM cases versus SM patients (33 vs. 14%, respectively; $p = 0.004$).

Allergy Study

Median (range) sBt levels in SM, c-sMCAD and nc-sMCAD cases were 26.3 $\mu\text{g/l}$ (6.8–515), 18.6 $\mu\text{g/l}$ (8.4–40.3) and 20 $\mu\text{g/l}$ (3.5–151), respectively. sBt levels $>25 \mu\text{g/l}$ were detected in 51% of SM and 26% of non-SM patients ($p = 0.001$), while sBt levels $<15 \mu\text{g/l}$ were more frequent among non-SM cases versus SM patients (31 vs. 11%, respectively; $p = 0.003$). Conversely, no significant ($p > 0.05$) differences in median total serum IgE levels were observed between both groups of patients (64.9 kU/l, range: 1–1,247 vs. 38 kU/l, range: 2–1,194, respectively). Overall, an IgE-mediated mechanism was detected in 70/142 (49%) cases, mainly among cases that were exclusively triggered by insects (46/56; 82%); these IgE-mediated cases more frequently corresponded to SM than non-SM patients (61 vs. 39%; $p = 0.01$), but no differences were observed when cases only triggered by insects were

excluded from the analysis; interestingly, among these latter cases, all subjects who showed a negative allergy study ($n = 10$) fulfilled criteria for SM.

REMA Score

Figure 1 shows the REMA score values obtained for the different diagnostic subgroups. ROC curve analyses confirmed that a REMA score ≥ 2 was the most efficient cut-off value to predict both MC clonality and SM (fig. 2); at this cutoff value, an area under the curve of 0.846 ($p < 0.001$) and 0.857 ($p < 0.001$) was observed, respectively (sensitivity/specificity of 84/74% and 87/73%, respectively). These results reflected a significantly ($p < 0.001$) higher frequency of cases with a REMA score ≥ 2 among patients with clonal versus those with non-clonal BM MC (83%, $n = 66/79$ vs. 26%, $n = 14/53$, respectively) and also among patients with SM versus those who did not fulfill the WHO criteria for SM (87%, $n = 70/80$ vs. 27%, $n = 21/78$, respectively). Once compared to the optimal sBt cutoff value obtained in our series (23.1 $\mu\text{g/l}$), a higher ef-

Fig. 1. Distribution of SM versus non-SM patients presenting with systemic MC mediator release symptoms according to the REMA score. ISM = Indolent SM.

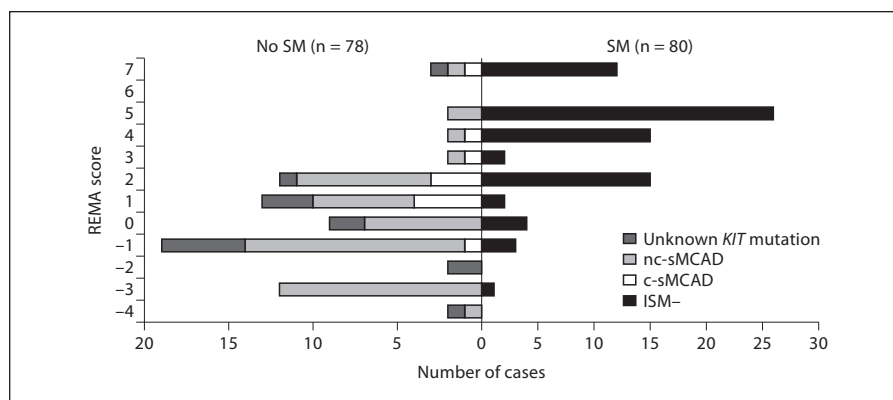
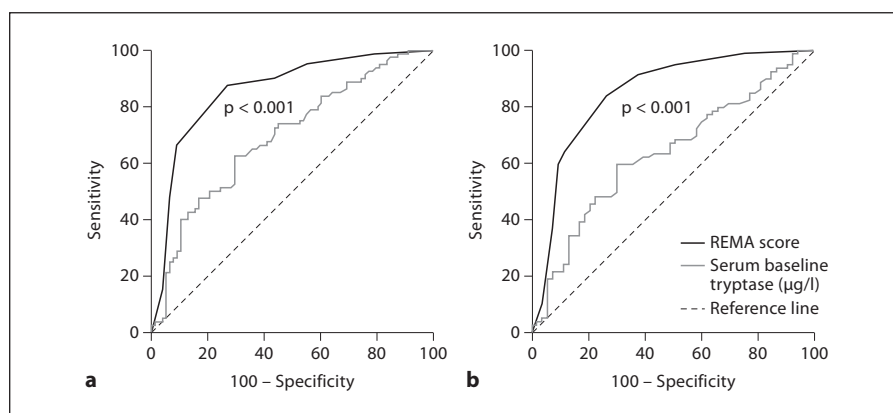


Fig. 2. ROC curve analyses of the efficiency (sensitivity, specificity and area under the curve) of the REMA score versus sBt levels alone to predict BM MC clonality (a) and SM (b) in patients presenting with systemic MC mediator release symptoms in the absence of MIS.



efficiency of the REMA score was observed, the former criteria showing a significantly ($p < 0.001$) lower area under the curve, sensitivity and specificity at this sBt cutoff level to predict both MC clonality (0.633, 59 and 70%, respectively) and the presence of SM (0.693, 62 and 71%, respectively) versus the REMA score (see text above and fig. 2).

Discussion

Given the clinical heterogeneity of patients presenting with systemic MC activation-related symptoms, their recognition and appropriate classification remain a challenge for physicians in daily clinical practice. In the present study, we have further investigated the clinical utility of the REMA score [3] in a larger prospective series of unselected patients to predict not only MC clonality but also the diagnosis of SM according to WHO criteria prior to a BM study. Overall, our results confirm that a REMA score ≥ 2 is associated with the highest sensitivity and specificity to predict both MC clonality and SM, the efficiency of this score-based approach being significantly higher than the

optimal sBt cutoff values (23.1 $\mu\text{g/l}$) obtained for the same purposes in the same group of patients. Accordingly, a REMA score ≥ 2 was able to predict MC clonality in most clonal cases and to correctly classify $>85\%$ of SM patients.

Interestingly, in our study, we have identified a group of patients who, despite having a clonal BM MC population (even in the context of an aberrant expression of CD25+ by BM MC in some of these cases) did not fulfill the WHO criteria for SM. Following recently proposed criteria, these patients were classified from a molecular point of view as c-sMCAD for a clear distinction from both SM cases and nc-sMCAD patients. At present, it is well known that somatic *KIT* mutations involving codon 816 are associated with constitutive downstream activation of MC, independent of stem cell factor binding; therefore, these *KIT* mutations in c-sMCAD cases may facilitate by themselves the development of MC activation symptoms although not enough criteria for SM are fulfilled. Whether these c-sMCAD cases actually correspond to early stages of true SM or whether they represent a new clonal MC-related entity remains to be determined and deserves further investigations.

In summary, in the present study, we confirm the clinical utility of the REMA score to be used prospectively to predict not only MC clonality, but also the presence of an underlying SM in patients presenting with systemic MC activation symptoms, including anaphylaxis. Although the REMA score cannot replace the application of the WHO-accepted criteria to diagnose or absolutely rule out SM, we strongly recommend considering this simple method to be applied by general practitioners and medical specialists as part of the workup of all patients presenting with systemic MC mediator release episodes in the absence of skin lesions for the screening of clonal MC disorders including c-sMCAD and SM. This would avoid unnecessary BM studies and, at the same time, it will improve the identification of SM cases lacking MIS who have low sBt levels. Given the typically low BM MC burden detected in most cases, it is recommended that BM studies are carried out in Reference Centers for MC-relat-

ed diseases, in which highly sensitive techniques are routinely applied to the study of the BM MC immunophenotype and molecular features (e.g. *KIT* mutation) at minimal disease states, to avoid false-negative results.

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