Original Article

Mast Cell Activation Syndromes: Comparison Between Two Scoring Models to Predict for Mast Cell Clonality

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What is already known about this topic? The National Institutes of Health idiopathic clonal anaphylaxis score (NICAS) and the Spanish Network on Mastocytosis score (REMAs) are used to screen for clonality in mast cell activation syndromes. The NICAS was validated for idiopathic anaphylaxis and showed greater accuracy than the REMAs.

What does this article add to our knowledge? The REMAs seems to be more accurate than the NICAS, particularly among men, and for systemic mastocytosis and anaphylaxis featuring urticaria and/or cardiovascular symptoms, as well as in patients with a blood-negative/bone marrow-positive *KIT* mutation, but not a hereditary α-tryptasemia—associated genotype.

How does this study impact current management guidelines? More sensitive blood-based molecular assays to detect *KIT*^{D816V} are needed. The combined use of the REMAs and blood detection of *KIT*^{D816V} is recommended to detect mast cell clonality.

BACKGROUND: The Red Española de Mastocitosis (Spanish Network on Mastocytosis) score (REMAs) and the National Institutes of Health idiopathic clonal anaphylaxis score (NICAS) were developed for more efficient screening of mast cell (MC) clonality in MC activation syndromes. In a limited idiopathic anaphylaxis case series, the NICAS showed higher accuracy compared with the REMAs.

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This study was supported by grants from the Instituto de Salud Carlos III (Madrid, Spain; Reference Nos. PI19/01166 and CIBERONC, CB16/12/00400), co-funded by the European Regional Development Funds (FEDER) (EQC2019-005419-P) within the Subprograma Estatal de Infraestructuras de Investigación y Equipamiento Científico Técnico de 2019 del Ministerio de Ciencia, Innovación y Universidades, Fundación Española de Mastocitosis (Madrid, Spain, FEM2021-SAM), Asociación Española de Mastocitosis y Enfermedades Relacionadas (AEDM-CTMC-2019) and Agencia Estatal de Investigación y Fondo Europeo de Desarrollo Regional. T.A.R. was supported by the 2019 European Academy of Allergy and Clinical Immunology Research Fellowship award. P.N.N. was supported by a grant from the Government of Castilla y León (Orden EDU 875 2021), Spain; and co-financed with the European Social Fund (BDNS (Identification No. 540787).
Conflicts of interest: The authors declare that they have no relevant conflicts of interest.
Received for publication August 2, 2022; revised October 24, 2022; accepted for publication November 30, 2022.
Available online December 16, 2022.
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2213-2198
© 2022 American Academy of Allergy, Asthma & Immunology
https://doi.org/10.1016/j.jaip.2022.11.042

Abbreviations used
ASO-qPCR- Allele-specific oligonucleotide quantitative polymerase chain reaction
BM- Bone marrow
BMM- Bone marrow mastocytosis
c-MCAS- Clonal mast cell activation syndrome
MC- Mast cell
MCAS- Mast cell activation syndrome
MMAS- Monoclonal mast cell activation syndrome not fulfilling diagnostic criteria for mastocytosis
MIS- Mastocytosis in the skin
NICAS- National Institutes of Health Idiopathic Clonal Anaphylaxis Score
nc-MCAS- Nonclonal mast cell activation syndrome including secondary plus idiopathic MCAS
REMA- Spanish Network on Mastocytosis (Red Española de Mastocitosis)
REMAs- Spanish Network on Mastocytosis (Red Española de Mastocitosis) score
sBT- Serum baseline tryptase
SM- Systemic mastocytosis

OBJECTIVE: To compare the performance of the REMAs against the NICAS in the diagnosis of MC clonality.
METHODS: We compared the diagnostic value of the REMAs against the NICAS in 182 patients (63% men, median age 56 years) who presented with anaphylaxis triggered by Hymenoptera venom allergy (45%), drugs (15%), food (11%), idiopathic anaphylaxis (20%), and mixed causes (10%). *KIT* mutation was assessed in parallel in whole blood and bone marrow (BM) and, when negative, in highly purified BM MC. *TPSAB1* was genotyped in a subset of 71 patients.
RESULTS: We found higher accuracy and rates of correctly classified patients for the REMAs (82% and 84%) compared with the NICAS (75% and 75%; $P = .02$ and $P = .03$, respectively), particularly among men ($P = .05$), patients with systemic mastocytosis ($P = .05$), those presenting anaphylaxis owing to any cause featuring urticaria ($P = .04$), cardiovascular symptoms ($P = .02$), and/or presyncope ($P = .02$) and those with a blood-negative/BM-positive *KIT* mutational profile ($P = .002$), but not hereditary α-tryptasemia−associated genotypes. Combined assessment of the REMAs and *KIT*^{D816V} in blood yielded an overall improved classification efficiency of 86% versus 84% for REMAs.
CONCLUSIONS: The combined use of the REMAs and blood detection of *KIT*^{D816V} is recommended, but more sensitive blood-based molecular assays to detect *KIT*^{D816V} are needed. **© 2022 American Academy of Allergy, Asthma & Immunology** **(J Allergy Clin Immunol Pract 2023;11:908-19)**

Key words: *Anaphylaxis; Mast cell activation syndromes; Mastocytosis;* KIT *mutation; Hereditary α-tryptasemia*

INTRODUCTION

Mast cell (MC) activation syndromes (MCAS) include a vast and heterogeneous group of patients who present with episodes of severe, systemic, and recurrent symptoms caused by proinflammatory and vasoactive mediators released by MCs, together with an increase in MC mediator (eg, tryptase) release, which usually respond to drugs that can counteract MC activation and/or the effects of MC mediators.[1] Patients with MCAS have been shown to more frequently carry a hereditary α-tryptasemia (HAT) genotype (an autosomal dominant genetic trait with variable penetrance associated with an increased copy number of the *TPSAB1* gene, which encodes for α-tryptase[2,3]) which is frequently associated with higher serum baseline tryptase (sBT) levels.[4,5] Based on recently proposed consensus criteria, MCAS can be classified into three major subtypes: (1) primary (ie, clonal) MCAS (c-MCAS), which include patients presenting with both MCAS and cutaneous mastocytosis (CM), systemic mastocytosis (SM), or monoclonal MCAS not fulfilling diagnostic criteria for mastocytosis (MMAS); (2) secondary MCAS (eg, resulting from allergies causing MCAS); and (3) idiopathic MCAS.[6] Although the clinical manifestations of HAT may vary from a complete lack of symptoms to severe and recurrent anaphylaxis,[2,3] patients with both SM and HAT (SM-HAT^{+}) appear to be at particularly higher risk for recurrent and severe anaphylaxis.[7]

In MCAS patients, MC activation may result from several pathophysiologic mechanisms,[8] which lead to anaphylaxis in a substantial fraction of them.[6] Recent data suggest that up to 5% of patients presenting with anaphylaxis may actually have an underlying primary MCAS, even when a well-established IgE-mediated mechanism is identified.[6,9] Thus, in some patients, primary MCAS may coexist with secondary MCAS (eg, SM associated with IgE-mediated Hymenoptera venom anaphylaxis [HVA]) or even idiopathic MCAS, which is defined as mixed (primary and secondary) MCAS.[1] To reach a final diagnosis of primary, secondary, idiopathic, or mixed MCAS, patients presenting with anaphylaxis may require a bone marrow (BM) study. Because of this and the relatively low prevalence of primary MCAS among all MCAS patients, the Spanish Network on Mastocytosis (REMA) proposed and validated a relatively simple and highly efficient score (REMAs) based on the patient's sex, clinical profile, and sBT levels to identify patients at risk for primary MCAS who should undergo further BM investigations.[10] A slightly modified version of this score, with lower cutoff values for sBT, rebranded as the Karolinska score, was applied to a small series of 30 idiopathic anaphylaxis patients, showing higher sensitivity and specificity.[11,12] More recently, the National Institutes of Health proposed another score to predict for MC clonality in patients presenting with idiopathic anaphylaxis.[13] The proposed National Institutes of Health idiopathic clonal anaphylaxis score (NICAS) added the *KIT*^{D816V} mutational status in blood, as assessed by allele-specific oligonucleotide quantitative polymerase chain reaction (ASO-qPCR), to the set of informative variables (slightly modified clinical findings and sBT levels) that had been included in the REMAs to identify MCAS patients at higher risk for having primary MCAS and who should undergo subsequent BM analyses.[13] When compared with the REMAs in a limited series of 56 patients, the NICAS algorithm showed a higher efficiency in terms of both sensitivity and specificity for detecting MC clonality in patients presenting with recurrent idiopathic anaphylaxis.[13] However, direct comparison between scores in a large series of patients with anaphylaxis triggered by well-established elicitors (in addition to patients with idiopathic anaphylaxis) is still missing.

Here, we compared the diagnostic accuracy of the REMAs versus the NICAS in a larger series of 182 patients referred to the REMA owing to anaphylaxis triggered by any cause, in the absence of mastocytosis in the skin (MIS), in whom a BM study was systematically performed.

METHODS

Study design

Between January 2018 and October 2020, we studied 182 consecutive patients referred to the REMA Reference Centers for MC disorders (Instituto de Estudios de Mastocitosis de Castilla La Mancha, Complejo Hospitalario Universitario de Toledo and Servício General de Citometria, NUCLEUS, Centro de Investigación del Cáncer, University of Salamanca) owing to anaphylaxis, in whom the KIT^{D816V} mutation was simultaneously assessed in whole blood and BM genomic DNA (gDNA). Inclusion criteria were strictly based on the suspicion of a primary MCAS by the referring physician, although 17% of referred patients presented with a negative REMAs (score of <2 points). The study was approved by the Ethics Committee of the Complejo Hospitalario Universitario de Toledo (Toledo, Spain). Before entering the study, all patients provided written informed consent to participate, in keeping with the Declaration of Helsinki.

A complete medical history and allergy workup was performed for every patient, including *in vivo* (skin tests and challenges) and/or *in vitro* (specific IgE for extracts and/or allergen molecular components) studies, according to the specific identified or suspected triggers. Based on the causes of anaphylaxis, patient triggers were divided into: 1) HVA; 2) drug-induced; 3) food allergy, including fish ingestion-related anaphylaxis owing to allergy to *Anisakis*; 4) idiopathic anaphylaxis; and 5) anaphylaxis induced by two or more triggers (mixed anaphylaxis). We measured sBT levels in every patient using the ImmunoCAP assay (Phadia/Thermo Fisher Scientific Inc, Uppsala, Sweden). The KIT^{D816V} mutation was systematically tested by ASO-qPCR in blood, as previously described (see Figure E1 in this article's Online Repository at http://www.jaci-inpractice.org).[14] Bone marrow studies were also performed in each patient, including 1) a careful cytomorphologic[15] and histopathologic examination of BM aspirated samples and BM core biopsies, respectively, carried out by two independent experts (I.A.-T. and M.M.)[16]; 2) an overall evaluation of BM cellularity and differential counts; 3) an in-depth immunophenotypic characterization of BM MC based on multiparametric flow cytometry[16]; and 4) a comprehensive molecular analysis of exon 17 *KIT* mutations (Figure 1).[17] The latter consisted of an initial screening for KIT^{D816V} in whole (unfractionated) BM by ASO-qPCR and, if negative, an assessment of that mutation (and other exon 17 *KIT* mutations) in fluorescence-activated cell sorting (FACS)-purified BM MC (purity of ≥97%), based on a peptide nucleic acid (PNA)-PCR technique, described elsewhere.[17] In addition, *KIT* sequencing was performed in a subset of 16 patients whose REMAs was 2 or more, and in which *KIT* mutations were not detected using these techniques.[18] *TPSAB1* genotyping was retrospectively performed in gDNA from a randomly selected subset of 71 patients, using a quantitative real-time digital PCR technique (Biomark, Fluidigm, San Francisco, Calif).[19]

The diagnosis of MCAS and SM was established according to World Health Organization 2017 criteria and recommendations,[20] whereas MMAS was defined as the presence of clonal BM MC (ie, codon 816 or otherwise *KIT*-mutated and/or CD25/CD2-positive BM MCs) in cases that did not fulfil criteria for SM.[1] For comparison, SM and MMAS patients were grouped under the term c-MCAS, whereas, in the absence of MC clonality, secondary and idiopathic MCAS patients were classified as nonclonal MCAS (nc-MCAS). The diagnosis of HAT was based on an increased *TPSAB1* copy number (ie, three or more α-tryptase gene copies or two α-tryptase gene copies in the presence of three β-tryptase gene copies) based on previously established criteria.[21]

For every patient, both the REMAs and NICAS were applied at patient referral based on clinical and laboratory features at that moment (Table I). For both models, a score value of 2 or more was considered to be associated with a higher probability of c-MCAS versus nc-MCAS, as previously indicated.[10,13] In addition, two new combined scores were calculated: 1) the combined REMAs and NICAS, in which scores of 2 or more for any of the two models were deemed positive; and 2) REMAs in combination with the presence versus absence of KIT^{D816V} in blood by ASO-qPCR, in which patients scoring 2 or more for the REMAs and/or those who tested positive for KIT^{D816V} in blood were deemed positive. To investigate the influence of HAT and *TPSAB1* gene copy number on these scores, we performed a correction of sBT levels for HAT by dividing the baseline tryptase level by 1 plus the number of extra α-tryptase gene copies, as proposed during the 2020 Working Conference on Mast Cell Disorders.[4]

Statistical methods

For all continuous variables, medians and range values were calculated, whereas frequencies were used for categorical parameters. Mann-Whitney U and χ^2 or Fisher exact tests were used to assess the statistical significance of differences observed between groups for continuous and categorical variables, respectively. Sensitivity and specificity, as well as positive predictive values (PPVs) and negative predictive values (NPVs) for each of the four score models (REMAs, NICAS, REMAs and NICAS combined, and REMAs and KIT^{D816V} in blood, by ASO-qPCR) were calculated for both the whole patient group and separately for cases classified according to the specific triggers for anaphylaxis: HVA, drug hypersensitivity, food allergy, idiopathic anaphylaxis, and anaphylaxis caused by two or more triggers (mixed anaphylaxis). Confidence intervals for these diagnostic values were calculated through the simple asymptotic method,[22] whereas differences between values obtained with each of the two scores were calculated using the McNemar test for binary matched-pairs. P less than .05 was considered to be associated with statistical significance. All statistical analyses were performed using SPSS for Windows (version 26.0, IBM Corporation, Armonk, NY).

RESULTS

Clinical features of c-MCAS versus nc-MCAS and HAT versus non-HAT cases

Of the 182 MCAS patients investigated, 115 (63%) were men and 67 (37%) were women; median age at referral was 55 years (range, 10-81 years). Except for a 10-year-old child, all other patients were adults (aged ≥18 years). Causes of anaphylaxis included HVA in 81 (45%) patients, unidentified triggers (idiopathic) in 36 (20%), drug hypersensitivity in 27 (15%), and food allergy in 20 (11%). The remaining 18 (10%) patients presented with two or more anaphylactic episodes caused by different triggers (mixed anaphylaxis). After BM analysis, 133 (73%) patients received the diagnosis of c-MCAS, 119 (65%) of whom had indolent SM and 14 (8%) of whom had MMAS. Moreover, 49 (27%) patients had nc-MCAS owing to normal BM cytologic, histopathologic, immunophenotypic, and molecular findings (Table I).

Overall, c-MCAS was found more frequently among men than women (68% vs 49%, respectively; $P = .02$), whereas no significant differences in age were observed between c-MCAS and nc-MCAS patients (median, 55 vs 58 years, respectively) (Table I). Concerning triggers of anaphylaxis, HVA was more

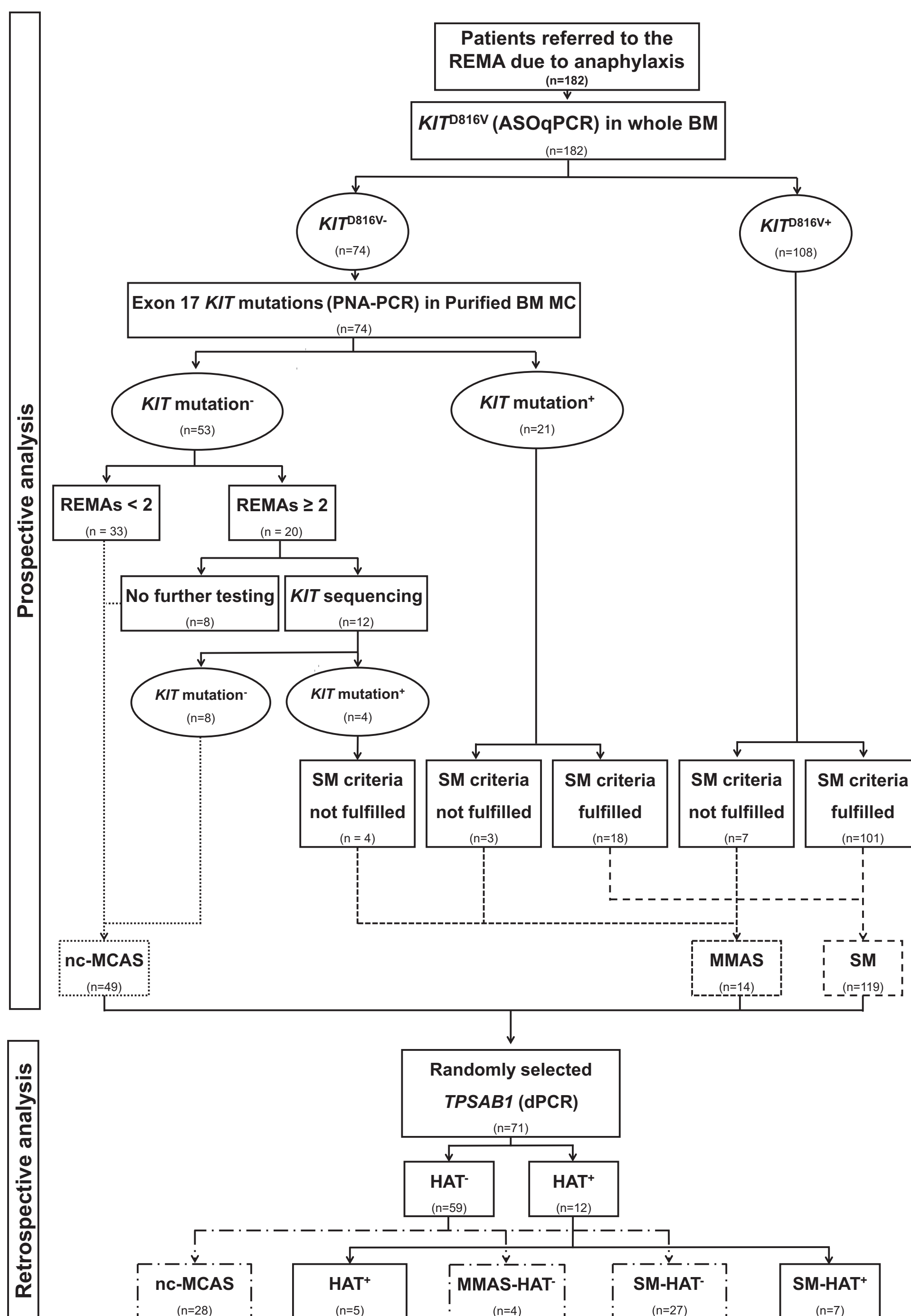

FIGURE 1. Flowchart illustrating how patients were evaluated during the prospective and retrospective analyses performed in this study, regarding bone marrow studies. *ASOqPCR*, allele-specific oligonucleotide quantitative polymerase chain reaction; *BM*, bone marrow; *dPCR*, digital polymerase chain reaction; $HAT^{+/-}$, positive/negative for hereditary α-tryptasemia, respectively; *MC*, mast cell; *MMAS*, monoclonal mast cell activation syndrome; *nc-MCAS*, nonclonal mast cell activation syndrome; *PNA-PCR*, peptide nucleic acid polymerase chain reaction; *REMA*, Red Española de Mastocitosis (Spanish Network on Mastocytosis); *SM*, systemic mastocytosis.

common among c-MCAS patients (50% vs 29%; $P = .01$), particularly within SM patients (54% vs 21% of MMAS cases, respectively; $P = .03$). In turn, drug hypersensitivity predominated ($P = .04$) among nc-MCAS (24%) versus c-MCAS (11%). No other significant differences were found between c-MCAS and nc-MCAS or between SM and MMAS regarding the frequency of idiopathic anaphylaxis, and anaphylaxis triggered by food allergy or mixed causes (Table I).

During anaphylactic episodes, patients with c-MCAS displayed cardiovascular symptoms (ie, presyncope and/or syncope) more frequently than did those with nc-MCAS (98% vs 73%, respectively; $P < .001$). Cardiovascular symptoms were

TABLE I. Clinical, demographic, and laboratory features of MCAS patients grouped according to the diagnostic subtype of disease

		MCAS			Clonal MCAS		
Variable	**Total (n = 182)**	**Nonclonal (n = 49)**	**Clonal (n = 133)**	**P**	**MMAS (n = 14)**	**SM (n = 119)**	**P**
Men	115 (74%)	24 (49%)	91 (68%)	**.02**	8 (57%)	83 (70%)	.22
Age, y	55 (10-80)	57 (10-77)	54 (24-80)	.31	61 (33-75)	55 (24-80)	**.03**
Triggers of anaphylaxis							
Hymenoptera	81 (45%)	14 (29%)	67 (50%)	**.01**	3 (21%)	64 (54%)	**.03**
Drugs	27 (15%)	12 (24%)	15 (11%)	**.04**	6 (43%)	10 (8%)	**<.001**
Idiopathic	36 (20%)	12 (24%)	24 (18%)	.32	4 (29%)	20 (17%)	.22
Food	20 (10%)	5 (10%)	15 (11%)	.55	0 (0%)	15 (13%)	.17
Mixed	18 (10%)	7 (14%)	11 (8%)	0.3	1 (7%)	10 (8%)	.92
Mucocutaneous symptoms	69 (38%)	34 (69%)	35 (26%)	**<.001**	6 (43%)	29 (24%)	.09
Urticaria	34 (19%)	23 (47%)	11 (8%)	**<.001**	4 (29%)	7 (6%)	**.004**
Pruritus	54 (30%)	26 (53%)	28 (21%)	**<.001**	5 (36%)	23 (19%)	.11
Angioedema	25 (14%)	10 (20%)	15 (11%)	.11	3 (21%)	12 (10%)	.16
Cardiovascular symptoms	166 (91%)	36 (73%)	130 (98%)	**<.001**	11 (79%)	119 (100%)	**<.001**
Presyncope	131 (72%)	29 (59%)	102 (77%)	**.02**	6 (43%)	96 (81%)	**.002**
Syncope (with or without presyncope)	124 (68%)	24 (49%)	100 (75%)	**.01**	5 (36%)	95 (80%)	**<.001**
Serum baseline tryptase, μg/L	16.8 (1.4-217)	9.6 (1.4-51.8)	18.6 (2.3-217)	**<.001**	14.2 (2.3-26.6)	20.8 (2.88-217)	**.01**
Serum baseline tryptase ≤11.4 μg/L	58 (32%)	26 (53%)	32 (24%)	**<.001**	6 (43%)	26 (22%)	.15
Serum baseline tryptase ≥25 μg/L	57 (31%)	8 (16%)	49 (37%)	**.008**	2 (14%)	47 (39%)	.09
KIT mutations	133 (73%)	0 (0%)	133 (100%)	**<.001**	14 (100%)	119 (100%)	1.00
KIT^{D816V} mutation	125 (69%)	0 (0%)	125 (94%)	**<.001**	10 (71%)	115 (97%)	**.02**
Whole PB^+	65 (36%)	0 (0%)	65 (49%)	**<.001**	1 (7%)	64 (54%)	**.001**
Whole BM^+	108 (59%)	0 (0%)	108 (81%)	**<.001**	7 (50%)	101 (80%)	**.02**
BM^+PB^+	65 (36%)	0 (0%)	65 (49%)	**<.001**	1 (17%)	64 (64%)	**.02**
BM^+PB^-	44 (24%)	0 (0%)	44 (33%)	**<.001**	5 (83%)	36 (36%)	**.02**
Purified BM MC^+ whole BM^-	17 (9%)	0 (0%)	17 (13%)	**<0.001**	3 (21%)	14 (12%)	.37
Other *KIT* mutations, purified BM MC (n = 16)*	8 (50%)	0 (0%)	8 (6%)	.22	4 (29%)	4 (3%)	**<.001**
Hereditary α-tryptasemia (n = 71)	12/71 (17%)	5/33 (15%)	7/38 (18%)	.71	0/4 (0%)	7/34 (21%)	.32
REMA score ≥ 2	137 (83%)	17 (35%)	120 (90%)	**<.001**	10 (71%)	110 (92%)	**.01**
NICAS ≥ 2	130 (71%)	21 (43%)	109 (82%)	**<.001**	8 (57%)	101 (85%)	**.004**

BM, Bone marrow; *MC*, mast cell; *MCAS*, mast cell activation syndrome; *MMAS*, monoclonal mast cell activation syndrome; *nc-MCAS*, nonclonal mast cell activation syndrome; *NICAS*, National Institute of Health idiopathic clonal anaphylaxis score; *PB*, peripheral blood; *REMA*, Red Española de Mastocitosis (Spanish Network on Mastocytosis); *SM*, systemic mastocytosis.

Results are expressed as the number of patients from all patients in the group and as percentages in parentheses (rounded to units) or as median values and ranges in parentheses. Significant differences among groups are highlighted in bold.

*Analyzed in 16 patients with REMA score of ≥2, with the results: KIT^{M541L} in four; KIT^{D816H} in two; KIT^{D816T} in one; and KIT^{D816A} in one patient.

also significantly more common in SM versus MMAS patients (100% vs 79%, respectively; $P < .001$) (Table I). In contrast, mucocutaneous symptoms (ie, pruritus, urticaria, and/or angioedema) were significantly more prevalent among nc-MCAS compared with c-MCAS patients (69% vs 26%, respectively; $P < .001$), with a tendency ($P = .09$) toward a higher frequency in MMAS (43%) versus SM (24%) patients (Table I). Interestingly, when specific mucocutaneous symptoms were analyzed separately, a higher frequency of urticaria and pruritus was observed among nc-MCAS versus c-MCAS patients (47% and 53% vs 8% and 21%, respectively; $P < .001$), with a similar frequency of angioedema in both patient groups ($P = .11$) (Table I).

Among patients presenting with HAT, we observed a 1:1 ratio of men to women and a similar median age to non-HAT patients (median, 57 years in both; $P = .93$) (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). In addition, no significant differences were found between HAT and non-HAT patients regarding triggers and symptoms occurring during anaphylaxis (Table E1). Moreover, the proportion of HAT cases was similar among c-MCAS versus nc-MCAS (15% vs 18%, respectively; $P = .71$) (Table I).

LABORATORY FEATURES OF C-MCAS VERSUS NC-MCAS

Median sBT levels were significantly higher in c-MCAS versus nc-MCAS patients (18.6 vs 9.6 μg/L, respectively; $P < .001$), and within c-MCAS, in SM versus MMAS patients (20.8 vs 14.2 μg/L, respectively; $P = .01$) (Table I). Although normal sBT levels (ie, ≤11.4 μg/L) were detected in patients with both c-MCAS and nc-MCAS, these were more frequently found among the latter group (24% vs 53%, respectively; $P < .001$). In turn, c-MCAS patients more frequently had sBT levels greater than 25

TABLE II. Diagnostic performance of the REMAs versus the NICAS algorithms for diagnosis of primary (clonal) mast cell activation syndrome grouped according to trigger for anaphylaxis and different sBT levels

Variable	Sensitivity	Specificity	PPV	NPV	Accuracy
REMAs					
Total patient cohort					
Primary mast cell activation syndrome (n = 182)	90 (85-95)	63 (49-76)	86 (80-92)	71 (58-84)	82*
Patient subgroup per trigger for anaphylaxis					
Hymenoptera (n = 80)	91 (84-98)	53 (28-79)	89 (82-97)	57 (31-83)	84
Idiopathic (n = 36)	92 (81-100)	92 (76-100)	96 (87-100)	85 (65-100)	92
Drugs (n = 28)	88 (71-100)	50 (22-78)	70 (50-90)	75 (45-100)	71
Food (n = 19)	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)	100
Mixed (n = 19)	75 (51-100)	43 (6-80)	69 (44-94)	50 (10-90)	63
Patient subgroup per sBT cutoff					
≤11.4 (n = 58)	84 (68-100)	69 (50-88)	77 (63-91)	78 (61-95)	78
<15 (n = 78)	83 (67-98)	72 (56-88)	81 (70-92)	74 (59-90)	78†
>20 (n = 74)	98 (89-100)	64 (30-97)	94 (88-100)	88 (65-100)	93
>25 (n = 57)	100 (100-100)	50 (1-99)	92 (85-100)	100 (100-100)	93
NICAs					
Total patient cohort					
Primary mast cell activation syndrome (n = 182)	82 (76-89)	57 (43-70)	83 (77-90)	56 (42-69)	75*
Patient subgroup per trigger for anaphylaxis					
Hymenoptera (n = 80)	85 (76-93)	60 (35-85)	90 (83-98)	47 (25-70)	80
Idiopathic (n = 36)	83 (68-98)	75 (51-100)	87 (73-100)	69 (44-94)	81
Drugs (n = 28)	81 (62-100)	25 (1-50)	59 (39-80)	50 (10-90)	57
Food (n = 19)	80 (60-100)	25 (0-67)	80 (60-100)	25 (0-67)	68
Mixed (n = 19)	67 (40-93)	86 (60-100)	89 (68-100)	60 (30-90)	74
Patient subgroup per sBT cutoff					
≤11.4 (n = 58)	66 (35-97)	65 (48-83)	70 (54-86)	61 (43-79)	66
<15 (n = 78)	70 (45-94)	565 (39-73)	70 (56-83)	56 (39-73)	64†
>20 (n = 74)	92 (68-116)	55 (25-84)	92 (85-99)	55 (25-84)	86
>25 (n = 57)	92 (65-119)	50 (15-85)	92 (84-100)	50% (15-85)	86

NICAS, National Institutes of Health Idiopathic Clonal Anaphylaxis Score; *NPV*, negative predictive value; *PPV*, positive predictive value; *REMAs*, Spanish Network on Mastocytosis score; *sBT*, serum baseline tryptase (μg/L).
Results are expressed as percentages (CIs).
*$P = .02$.
†$P = .05$.

μg/L compared with nc-MCAS patients: 37% versus 16%, respectively ($P < .001$) (Table I).

The KIT^{D816V} mutation was detected in 125 of 133 (94%) c-MCAS patients, of whom 65 of 133 (49%) tested positive in both whole blood and BM gDNA, and 44 of 133 (33%) had a positive KIT^{D816V} mutation only in whole BM gDNA whereas it was negative in blood. Another 17 of 133 (13%) patients had a positive test for KIT^{D816V} in purified BM MC samples only (Table I). Whole BM-positive/blood-negative as well as purified BM MC-positive/whole BM-negative cases were more frequently found among MMAS versus SM patients (83% vs 36%, $P = .02$; and 21% vs 12%, $P = .37$, respectively). Other *KIT* mutations involving exon 17 were detected by PNA-PCR analysis of FACS-purified BM MC in four SM patients who had tested negative in unfractionated whole BM by ASO-qPCR: KIT^{D816H} was found in two patients whereas KIT^{D816T} and KIT^{D816A} variants were found in one patient each. In addition, direct sequencing of PCR products obtained from FACS-purified BM MC revealed the M451L polymorphism in the transmembrane domain of the *KIT* gene (exon 10) in four MMAS patients.

In contrast to the *KIT* mutation, genotypes consistent with HAT were found in five of 33 (15%) nc-MCAS patients and seven of 38 (18%) c-MCAS patients; all of the latter cases corresponded to SM (seven of 34; 21%). Except for the 0α4β genotype that was present in 10 of 33 (30%) nc-MCAS patients versus three of 38 (8%) c-MCAS patients ($P = .03$), no statistically significant differences were found in the frequency of all other HAT genotypes identified among the different groups of patients. Briefly, HAT 2α3β was the most frequent genotype, which was present in four of 33 (12%) nc-MCAS patients and three of 38 (8%) c-MCAS patients, followed by the 3α2β genotype found in one of 33 (3%) nc-MCAS patients and one of 38 (3%) c-MCAS patients. The 3α3β (two of 38; 5%) and 3α4β (one of 38; 3%) HAT genotypes were restricted in our series to a small percentage of c-MCAS patients (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). Overall, MCAS patients who had a HAT genotype had significantly higher sBT levels than those who were HAT-negative (26 vs 15.3 μg/L, respectively; $P = .011$), whereas median sBT levels did not differ significantly between patients with HAT who had a diagnosis of nc-MCAS versus c-MCAS (22.5 vs 27.3 μg/L, respectively; $P = .34$) (Table E2). In contrast, among HAT-negative patients, those with c-MCAS had significantly higher sBT levels compared with those with nc-MCAS (23.3 vs

6.2 μg/L, respectively; $P < .001$) (Table E2). Among the 71 patients investigated for HAT genotypes, three non-HAT/nc-MCAS patients had an sBT greater than 25 μg/L. None of them had renal dysfunction and the clinical histories were unremarkable except for (1) recurrent urticaria with sensitization to *Anisakis*, (2) recurrent flushing, and (3) congenital amegakaryocytic thrombocytopenia with no signs of myelodysplasia, respectively.

Comparison of REMAs and NICAS for c-MCAS

Overall, we found a REMAs and NICAS of 2 or greater in 137 (75%) patients and 130 (71%) patients, respectively (Table I). The percentage of cases above this cutoff value was significantly higher among c-MCAS compared with nc-MCAS patients for both the REMAs (90% vs 35%; $P < .001$) and the NICAS (80% vs 43%; $P < .001$). When we compared the performance of both scores in the whole patient cohort, the REMAs showed a slightly higher sensitivity (90% vs 82%) and specificity (63% vs 57%), which was associated with both a higher PPV (86% vs 83%) and NPV (71% vs 56%). These results translated into a significantly higher accuracy for the REMAs versus NICAS: 82% versus 75% ($P = .02$). Similar results were observed when we divided patients according to different triggers for anaphylaxis and separately considered HVA (91% vs 85%), idiopathic (92% vs 83%), and food (100% vs 80%) triggered anaphylaxis patient subgroups, but differences did not reach statistical significance ($P > .05$). In turn, the specificity of the REMAs was higher than that of the NICAS in the whole group as well as in all trigger categories, except for HVA (53% vs 60%), and particularly for patients with mixed anaphylaxis (43% vs 86%) in the absence of statistically significant differences (Table II).

When patients were grouped according to the different sBT cutoff levels, the REMAs showed a higher sensitivity, specificity, and accuracy than the NICAS in every tryptase range (ie, ≤11.4, <15, >20, and >25 μg/L) (Table II).

Overall, 152 of 182 (84%) patients were correctly classified by the REMAs and 137 of 182 (75%) by NICAS ($P = .03$), including 92% of SM, 71% of MMAS, and 65% of nc-MCAS patients, who were correctly classified with the REMAs versus 85% ($P = .05$), 57% ($P = .46$), and 57% ($P = .38$) with NICAS, respectively (Table III). The presence of HAT genotypes was associated with misclassification of two of 12 (17%) patients and three of 12 (25%) patients with the REMAs and the NICAS, respectively ($P = .69$) (Table III). The two patients misclassified with the REMAs included 1) a woman with an sBT value of 21.4 μg/L and a 2α3β *TPSAB1* genotype, who had HVA-triggered and idiopathic episodes of anaphylaxis presenting with angioedema and syncope, who scored 0 with the REMAs while having SM; and 2) a man with an sBT level of 33.1 μg/L and a 3α2β *TPSAB1* genotype, who had HVA-triggered anaphylaxis presenting with urticaria, angioedema, and presyncope, and who scored 4 with the REMAs while having an nc-MCAS. In turn, the three patients misclassified with NICAS consisted of: 1) a man with 27.3 μg/L sBT and a 3α4β *TPSAB1* genotype, who had idiopathic anaphylaxis presenting with flushing and presyncope and whose *KIT* mutational profile was positive in purified BM MC while negative in whole BM and peripheral blood, and who scored 1 with NICAS while having SM; 2) a man with an sBT level of 18.2 μg/L and a 3α2β *TPSAB1* genotype, who had food-triggered anaphylaxes presenting with flushing, dyspnea, and presyncope and whose *KIT* mutational profile was negative in blood (and positive for KIT^{D816H} in purified BM MC), who scored 1 with the NICAS while having SM; and 3) a woman with 22.5 μg/L sBT and a 2α3β *TPSAB1* genotype, who had drug-triggered anaphylaxis presenting with generalized pruritus and syncope, who tested negative for the KIT^{D816V} and for other *KIT* mutations, and who scored 3 with the NICAS while having an nc-MCAS. The REMAs was also more accurate in classifying patients with different clinical manifestations of anaphylaxis, such as those presenting with urticaria (88% vs 65%; $P = .04$), cardiovascular symptoms (84% vs 70%; $P = .02$), and presyncope (84% vs 73%; $P = .02$). Importantly, the accuracy of the REMAs was also higher than that of the NICAS in identifying c-MCAS patients who showed the KIT^{D816V} mutation in BM while it was undetected in whole blood (79% vs 62%; $P = .007$), including both those with negative KIT^{D816V} in whole blood while positive in whole BM (79% vs 61%; $P = .002$), and those with the KIT^{D816V} mutation restricted to BM MC (100% vs 57%). In turn and besides these higher diagnostic values, a tendency toward a better classification was also observed for the REMAs versus NICAS for all tryptase ranges (ie, ≤11.4, <15, >20, and >25 μg/L) as well as for all individual elicitors of anaphylaxis (except for those for whom anaphylaxis was triggered by mixed causes). Table III lists the main demographic, clinical, and laboratory characteristics of patients that were correctly classified according to the REMAs and the NICAS.

Among the 45 cases misclassified by the NICAS, 31 (69%) were correctly classified by the REMAs, whereas 16 of 30 (53%) patients misclassified by the REMAs were well-classified with the NICAS.

After applying the aforementioned correction for HAT, the proportion of correctly classified cases decreased slightly with both the REMAs (one of 71 previously correctly classified cases became misclassified) and the NICAS (two of 71 previously correctly classified cases became misclassified, and one of 71 cases previously incorrectly classified became correctly classified). A detailed description of cases that were modified in classification when sBT values were corrected by the HAT genotype is provided in the Supplemental Results in this article's Online Repository at http://www.jaci-inpractice.org).

Combined REMAs plus NICAS and REMA plus KIT^{D816V} mutation in blood scores

Based on the findings above, we evaluated the potential utility of combining the REMAs and the NICAS, and the REMAs combined with the KIT^{D816V} mutational status in blood by ASO-qPCR and compared the performance of the two new models with the REMAs and the NICAS. Considering the performance of the REMAs/NICAS versus the REMAs, we found a higher sensitivity (96% vs 90%) and NPV (80% vs 71%), whereas both the specificity (41% vs 63%) and PPV (82% vs 86%) were lower, translating into a nonsignificant and slightly lower accuracy for the REMAs/NICAS (81% vs 82% for the REMAs; $P = .53$). Similar results were found for the different triggers for anaphylaxis (except for HVA and mixed-caused anaphylaxis, in which the REMAs/NICAS showed a higher accuracy) and for the different sBT cutoff levels (Table IV). In turn, the REMAs/KIT^{D816V} in blood model yielded a higher sensitivity (94% vs 90%), specificity (65% vs 63%), PPV (88% vs 86%), and NPV (80% vs 71%), translating into a tendency toward a higher accuracy (86% vs 82%;

TABLE III. Clinical, demographic, and laboratory features of MCAS patients correctly classified by REMAs compared with NICAS, combined REMAs and NICAS score, and combined REMAs and whole blood KIT^{D816V} mutational status

Patient group	REMAs	NICAS	*P*	REMAs$^+$ or NICAS$^+$	*P*	REMAs$^+$ or KIT^{D816V} PB$^+$	*P*
Correctly classified patients (n = 182)	152 (84%)	137 (75%)	**.02**	148 (81%)	.53	157 (86%)	.06
Misclassified by REMAs (n = 30)	—	16 (53%)	NA	8 (27%)	NA	5 (17%)	NA
Positive score	120/137 (88%)	109/130 (80%)	NA	128/157 (82%)	NA	125/142 (88%)	NA
Sex							
Women (n = 67)	53 (79%)	48 (72%)	.26	55 (82%)	.69	56 (84%)	.25
Men (n = 115)	99 (86%)	89 (77%)	**.05**	93 (81%)	.18	101 (88%)	.5
Diagnosis							
SM (n = 119)	110 (92%)	101 (85%)	**.05**	117 (98%)	**.03**	115 (96%)	0.13
SM HAT$^+$ (n = 7)	6 (86%)	5 (71%)	.63	7 (100%)	NC	6 (86%)	1.00
SM HAT$^-$ (n = 27)	27 (100%)	23 (85%)	NC	27 (100%)	1.0	27 (100%)	1.00
MMAS (n = 14)	10 (71%)	8 (57%)	.46	12 (86%)	.5	11 (79%)	1.00
MMAS HAT$^-$(n = 4)	3 (75%)	2 (50%)	.50	3 (75%)	1.00	3 (75%)	1.00
nc-MCAS (n = 49)	32 (65%)	28 (57%)	.38	20 (41%)	**<.001**	32 (65%)	1.00
nc-MCAS HAT$^-$ (n = 5)	4 (80%)	4 (80%)	.50	3 (60%)	1.00	4 (80%)	1.00
nc-MCAS HAT$^-$ (n = 28)	18 (64%)	16 (57%)	.55	12 (43%)	**.03**	18 (64%)	1.00
HAT (n = 12)	10 (83%)	9 (75%)	.69	10 (83%)	1.00	10 (83%)	1.00
Triggers for anaphylaxis							
HVA (n = 80)	68 (85%)	65 (81%)	.48	70 (88%)	.69	71 (89%)	.25
Idiopathic (n = 36)	33 (92%)	29 (81%)	.13	30 (83%)	.25	33 (92%)	1.00
Drug hypersensitivity (n = 28)	20 (71%)	16 (57%)	.22	18 (64%)	.69	21 (75%)	.25
Food (n = 19)	19 (100%)	13 (68%)	NC	16 (84%)	NC	19 (100%)	1.00
Mixed (n = 19)	12 (63%)	14 (74%)	.5	14 (74%)	.5	14 (74%)	.5
Clinical manifestations							
Mucocutaneous symptoms (n = 69)	52 (75%)	48 (70%)	.5	48 (70%)	.5	57 (83%)	.06
Urticaria (n = 34)	30 (88%)	22 (65%)	**.02**	22 (65%)	**.02**	30 (88%)	1.00
Angioedema (n = 25)	16 (64%)	19 (76%)	.26	20 (80%)	.29	19 (76%)	.25
Pruritus (n = 54)	42 (78%)	37 (69%)	.26	37 (69%)	.3	46 (85%)	.13
Cardiovascular symptoms (n = 166)	139 (84%)	124 (75%)	**.03**	136 (81%)	1.00	145 (87%)	1.00
Presyncope (n = 131)	110 (84%)	95 (73%)	**.02**	106 (80%)	.27	114 (86%)	.25
Syncope (n = 124)	105 (85%)	99 (80%)	.17	103 (82%)	.61	110 (88%)	.13
Laboratory findings							
sBT, μg/L	18.1 (1.4-217)	18.5 (2.4-217)	NA	18.2 (2.3-217)	NA	17.5 (1.4-217)	NA
sBT ≤11.4 (n = 58)	45 (78%)	38 (66%)	.17	43 (74%)	.75	48 (83%)	.25
sBT <15 (n = 78)	61 (78%)	50 (64%)	**.05**	56 (72%)	.3	65 (83%)	.13
sBT >20 (n = 74)	69 (93%)	64 (87%)	.11	68 (92%)	1.00	69 (93%)	1.00
sBT >25 (n = 57)	53 (93%)	49 (86%)	.13	52 (91%)	1.00	53 (93%)	1.00
KIT mutations (n = 133)	121 (90%)	109 (81%)	**.02**	129 (96%)	**.008**	126 (94%)	.06
KIT^{D816V} mutation$^+$ (n = 125)	115 (91%)	104 (82%)	**.03**	122 (97%)	**.02**	120 (95%)	.06
Whole PB$^-$ (n = 117)	92 (79%)	71 (60%)	**.001**	83 (70%)	**.04**	92 (79%)	1.00
Whole BM$^+$ (n = 108)	98 (90%)	93 (85%)	.26	105 (96%)	**.02**	103 (94%)	.06
BM$^+$/PB$^+$ (n = 65)	60 (92%)	65 (100%)	NC	65 (100%)	NC	65 (100%)	NC
BM$^+$/PB$^-$ (n = 44)	38 (85%)	28 (64%)	**.007**	40 (91%)	.5	38 (85%)	1.00
Purified BM MC$^+$/whole BM$^-$ (n = 17)	17 (100%)	11 (65%)	NC	17 (100%)	1.00	17 (100%)	1.00
Other *KIT* mutations$^{+\ -}$ purified BM MC* (n = 8)	6 (75%)	5 (63%)	.63	7 (88%)	1.00	6 (75%)	1.00

BM MC, Bone marrow mast cell; *c-MCAS*, clonal mast cell activation syndrome; *HAT*, hereditary α-tryptasemia; *HVA*, Hymenoptera venom allergy; *MCAS*, mast cell activation syndrome; *MMAS*, monoclonal mast cell activation syndrome; *NA*, not applicable; *NC*, not computable; *nc-MCAS*, non- clonal mast cell activation syndrome; *NS*, not statistically significant; *PB*, peripheral blood; *REMAs*, Spanish Network on Mastocytosis score; SM, systemic mastocytosis.

Results are expressed as the number of patients from all patients in the group and percentage in parentheses (rounded to units). *P* values refer to comparisons with REMAs. Statistically significant differences among groups are highlighted in bold.

*In these cases, the KIT^{D816V} mutation was positive as assessed by peptide nucleic acid polymerase chain reaction in fluorescence-activated cell sorting−purified BM MCs, but negative by allele-specific oligonucleotide quantitative polymerase chain reaction in both whole blood and whole bone marrow.

P = .06). The same results occurred for HVA, drug-triggered, and mixed-caused anaphylaxis as well as for the 11.4 or less and less than 15 μg/L sBT cutoffs, but not for idiopathic and food-allergy-triggered anaphylaxis, and for the greater than 20 and greater than 25 μg/L sBT cutoffs, for which diagnostic values were the same as those found for the REMAs (Table IV). Compared with the individual NICAS, both the combined REMAs/NICAS and REMAs/*KIT*^{D816V} models showed a higher sensitivity (82% vs 96% and 94%, respectively) and NPV (56% vs 80% and 80%, respectively), whereas the REMAs/NICAS showed a lower specificity (57% v. 41%) and PPV (83% vs 82%) and the REMA/*KIT*^{D816V} was associated with both a higher specificity (57% vs 65%) and PPV (88% vs 82%). These data translated into an overall accuracy of 81% for the combined REMAs/NICAS and of 86% for REMAs/*KIT*^{D816V} combined scores.

Thus, the use of REMAs/NICAS resulted in an overall decrease in correctly classified cases (81%) compared with both the individual REMAs (84%; P = .53) and the NICAS (75%; P = .04). In turn, use of the REMAs combined with the *KIT*^{D816V} assessment in blood was associated with a tendency toward an overall improved accuracy (86%) versus the REMAs (81%; P = .06) (Table III) and versus the NICAS (75%; P = .002).

Compared with the REMAs, the REMAs/NICAS was more accurate in classifying SM cases (98% vs 92%; P = .03), in patients with clonal disease/*KIT* mutations (96% vs 90%; P = .008), in those in whom the *KIT*^{D816V} mutation was detected (97% vs 91%; P = .02), and in those with the *KIT*^{D816V} mutation in whole BM (96% vs 90%; P = .04). In turn, this combined score was significantly less accurate in classifying nc-MCAS in general (41% vs 65%; P < .001), particularly for nc-MCAS/HAT-positive patients (43% vs 64%; P = .03), patients whose anaphylaxes presented with urticaria (65% vs 88%; P = .02), and patients with a negative whole blood *KIT*^{D816V} mutational profile (70% vs 79%; P = .04) (Table III). In turn, the new REMAs/*KIT*^{D816V} score showed a tendency toward an improved classification of patients who presented with mucocutaneous symptoms during anaphylaxis (83% vs 75%; P = .06), those with clonal disease/*KIT* mutations (94% vs 90%; P = .06), and especially those who had a *KIT*^{D816V} mutation (95% vs 91%; P = .06) (Table III).

DISCUSSION

According to widely accepted diagnostic criteria for MCAS, patients who experience severe and recurrent clinical manifestations indicative of MC activation (ie, MCAS) would need to undergo a complete BM study to confirm or rule out a primary (clonal) versus secondary/idiopathic or mixed MCAS.[4] Despite this, diagnostic screening of c-MCAS (ie, SM and MMAS) among patients presenting with systemic MC-mediator–related symptoms in the absence of MIS remains challenging. This is because only a minority of MCAS patients ultimately fulfill diagnostic criteria for SM or MMAS. Because of this (and the invasiveness of BM studies), BM aspiration and biopsy are not performed in many MCAS patients, or they are delayed. This results in the lack of a definitive diagnosis, or even a misdiagnosis, in a significant fraction of MCAS patients.

Over the past decade, scoring models have been proposed to identify MCAS patients at risk for primary MCAS, in which BM analyses are indicated, and to avoid unnecessary BM studies in secondary/idiopathic MCAS patients. Here, we compared the utility of the REMAs versus the NICAS in the diagnostic workup of a large series of MCAS patients presenting with anaphylaxis. Overall, our results showed a higher accuracy for the REMAs versus the NICAS in patients presenting with urticaria, cardiovascular symptoms, and presyncope, and in SM patients who tested negative for *KIT*^{D816V} in whole blood while being positive in BM, in addition to SM independently of sBT levels, owing to both greater sensitivity and specificity.

These results confirm the high accuracy of both the REMAs and NICAS in the diagnostic screening of MCAS patients with BM involvement by clonal MC (ie, primary MCAS). However, one-fourth (NICAS) and one-fifth (REMAs) of patients would be misclassified by these screening algorithms, in which 14% and 17% of primary MCAS cases would be wrongly regarded as (potential) secondary/idiopathic nc-MCAS not requiring a diagnostic BM study. Altogether, these results point to the need for more accurate biomarkers to identify patients at higher risk for c-MCAS in whom a diagnostic BM is indicated. This prompted us to compare the demographic, clinical, laboratory, and molecular features of c-MCAS versus nc-MCAS patients. Through this analysis, we confirmed the direct association of c-MCAS with male sex, HVA, cardiovascular symptoms (presyncope and syncope), higher sBT levels, and, as expected, the presence of *KIT*^{D816V} (and other *KIT* mutations) in blood and BM, as well as the greater frequency of REMAs and NICAS of 2 or greater. In contrast, nc-MCAS patients more frequently showed drug-induced anaphylaxis and mucocutaneous symptoms (ie, urticaria and pruritus) associated with lower (<2) REMAs and NICAS. Overall, these differences among c-MCAS versus nc-MCAS patients were even more pronounced within the former patients with SM than MMAS patients.

These findings support previous observations regarding to the lower frequency of anaphylaxis and particularly HVA among indolent SM patients presenting with skin lesions versus BM mastocytosis (BMM) and MMAS cases.[23-25] The former showed a greater prevalence of anaphylaxis caused by drug hypersensitivity and food allergy.[25,26] Previous studies showed that c-MCAS patients tend to be predominantly male and more prone to anaphylaxis presenting with cardiovascular findings (eg, presyncope, hypotension, shock, and cardiac arrest[24]), as was found here. In line with these results, the REMAs for predicting clonality[23] includes both features in its scoring model for c-MCAS, whereas NICAS scores positively only for patients who featured syncope (but not presyncope) during anaphylaxis,[13] even though presyncope is a well-established surrogate marker for hypotension.[27] In the current cohort, presyncope not followed by syncope was present in almost half of all MMAS patients and in one-fifth of SM patients, including those who did not display features compatible with a low MC burden (ie, low sBT levels and the *KIT* mutation restricted to BM MC). Inclusion of presyncope as a predictive marker for c-MCAS in the REMAs, but not the NICAS, might contribute to explaining, at least in part, the lower accuracy of the later scoring model.

In the study that led to the REMAs, patients presenting with urticaria and/or angioedema during anaphylaxis were five times less prone to having an underlying clonal MC disease, and thus to be diagnosed with nc-MCAS.[23] This was subsequently confirmed in a larger series of 158 MCAS patients presenting without MIS (80 SM and 78 nc-MCAS).[10] In turn, NICAS included urticaria as a feature associated with idiopathic

TABLE IV. Diagnostic performance of the combined REMAs and NICAS score, and the combination of REMAs and KITD816V mutation in blood, by ASO-qPCR (panel B) algorithms for the diagnosis of primary (clonal) MCAS grouped according to the trigger for anaphylaxis and different serum baseline tryptase levels

Variable	Sensitivity	Specificity	PPV	NPV	Accuracy	*P*
REMAs-positive or NICAS-positive						
Total patient cohort						
Primary MCAS (n = 182)	96 (93-99)	41 (27-55)	82 (76-88)	80 (64-96)	81	.53
Patient subgroup per trigger for anaphylaxis						
Hymenoptera (n = 80)	97 (93-100)	43 (17-69)	89 (82-96)	75 (45-100)	88	.69
Idiopathic (n = 36)	92 (81-100)	67 (40-93)	85 (71-98)	80 (55-100)	83	.25
Drugs (n = 28)	100 (100-100)	17 (0-38)	62 (43-80)	100 (100-100)	64	.69
Food (n = 19)	100 (100-100)	25 (0-67)	83 (66-100)	100 (100-100)	84	NC
Mixed (n = 19)	92 (78-100)	43 (6-80)	75 (54-96)	75 (33-117)	75	.5
Patient subgroup per sBT cutoff						
sBT ≤11.4 (n = 58)	97 (91-100)	46 (27-65)	69 (55-82)	92 (78-100)	74	.75
sBT <15 (n = 78)	93 (86-100)	41 (24-58)	69 (58-81)	81 (62-100)	72	.3
sBT >20 (n = 74)	100 (100-100)	45 (16-75)	91 (85-98)	100 (100-100)	92	1.00
sBT >25 (n = 57)	100 (100-100)	38 (4-71)	91 (83-98)	100 (100-100)	91	1.00
REMAs-positive or *KIT*^{D816V} peripheral blood-positive						
Total patient cohort						
Primary MCAS (n = 182)	94 (90-98)	65 (52-79)	88 (83-93)	80 (68-92)	86	.06
Patient subgroup per trigger for anaphylaxis						
Hymenoptera (n = 80)	95 (90-100)	57 (31-83)	91 (85-98)	73 (46-99)	89	.25
Idiopathic (n = 36)	92 (81-100)	92 (76-100)	96 (87-100)	85 (65-100)	92	1.00
Drugs (n = 28)	94 (82-106)	50 (22-78)	71 (52-91)	86 (60-100)	75	.25
Food (n = 19)	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)	100	1.00
Mixed (n = 19)	83 (62-100)	43 (6-80)	71 (48-95)	60 (17-100)	68	.5
Patient subgroup per sBT cutoff						
sBT ≤11.4 (n = 58)	96 (93-99)	41 (27-55)	82 (76-88)	80 (64-96)	83	.25
sBT <15 (n = 78)	92 (81-100)	67 (40-93)	85 (71-98)	80 (55-100)	83	.13
sBT >20 (n = 74)	98 (95-100)	64 (35-92)	94 (88-100)	88 (65-100)	93	1.00
sBT >25 (n = 57)	100 (100-100)	50 (15-85)	92 (85-100)	100 (100-100)	93	1.00

MCAS, Mast cell activation syndrome; NC, not computable; *NICAS*, National Institutes of Health Idiopathic Clonal Anaphylaxis Score; *NPV*, Negative predictive value; *PPV*, positive predictive value; *REMAs*, Spanish Network on Mastocytosis score; *sBT*, serum baseline tryptase (μg/L).
Results are expressed as percentages (CIs). *P* values refer to comparisons with the REMAs.

anaphylaxis in primary MCAS patients. This may be due to the limited number of c-MCAS patients included in this study (n = 8; four SM and four MMAS), and because virtually all reported anaphylactic reactions in this series (91%) featured urticaria. Here, we confirmed the association of urticaria (and pruritus) with nc-MCAS, but not with primary MCAS. Based on these findings, the inclusion of urticaria as a marker of c-MCAS in the NICAS might also contribute, at least partially, to the lower accuracy of the NICAS versus the REMAs reported in the current study.

KIT^{D816V} and other *KIT* mutations have long been shown to promote autophosphorylation and/or dimerization of the *KIT* receptor in a ligand-independent way. Thus, they may enhance cell proliferation and survival[28] as well as FcεRI-dependent MC degranulation.[29] Simultaneously, these *KIT* mutations decrease the threshold for MC activation induced by triggers that involve other pathways.[30] The availability of highly sensitive ASO-qPCR techniques for the detection of *KIT*^{D816V} has led to an increased rate of detection of the mutation in blood.[31] Its use is currently recommended in routine diagnostic screening for clonal MC disorders, particularly in patients presenting with MCAS,[4] in line with what is proposed by the NICAS. However, here we show that in more than half of all MCAS patients with *KIT*-mutated BM MC, the mutation went undetected in blood, and frequently even in whole BM gDNA. This limited sensitivity of the *KIT*^{D816V} assay in blood might further contribute to the lower accuracy of the NICAS versus the REMAs, reported in this study, as less than half of c-MCAS patients, including less than 10% of MMAS patients, tested positive for *KIT*^{D816V} in blood. The ASO-qPCR technique for detecting *KIT*^{D816V} in blood has been used to exclude MC clonality in patients with suspected MCAS.[32] However, previous studies, whose findings are in line with ours, have shown a limited sensitivity for this technique when applied to blood, as 6% to 19% of all SM patients, and up to one-third to more than 50% of all BMM cases presenting with MCAS without skin lesions (mostly including BMM patients with HVA)[33] who carry the *KIT*^{D816V} mutation in BM, tested negative in blood.[14,31,34,35] Although this technique should not be used alone to exclude MC clonality, our results show that combining the REMAs with the *KIT*^{D816V} assay results in blood translated into a tendency toward higher diagnostic accuracy compared with the REMAs alone.

Nevertheless, there were two main limitations to our study. First, except for the 10-year-old child, all of the patients were adults. Thus, our results might not be generalizable to pediatric patients. Second, the cohort was based on patients who were

referred to a reference center for MC disorders according to the referring physicians' criteria, which might have induced a biased selection of patients. In this regard, it should be noted that, 6% to 8% of individuals in the general population display elevated sBT,[36,37] whereas around half of the nc-MCAS patients in the current study had sBT levels greater than 11.4 μg/L, which suggests a selection bias. Because of this, our results warrant further confirmation in a (nonspecialized) allergy department setting.

Several diseases apart for primary MCAS have been associated with elevated sBT levels, which might be either related or not to MC activation, such as chronic spontaneous urticaria,[38] eosinophilic esophagitis,[39] severe chronic kidney disease,[40] and myeloid neoplasms[41] (eg, myelodysplastic syndromes, acute myeloblastic leukemia, and other myeloproliferative neoplasms). More recently, a dominantly inherited elevation of sBT, resulting from germ line multiplications of the *TPSAB1* gene (HAT) was described,[21] which might be associated with a higher risk for anaphylaxis.[42] However, here we showed a similar frequency of HAT in c-MCAS versus nc-MCAS patients, which might explain why the presence of HAT did not influence the accuracy of the two scores compared in this study. In fact, using HAT-corrected sBT levels to calculate the scores of individual MCAS patients slightly (nonsignificantly) decreased the accuracy of both the REMAs and the NICAS. However, future studies in larger cohorts of MCAS patients are needed to confirm our findings regarding the limited relevance of HAT-corrected scores.

In this study we compared for the first time the accuracy of REMAs versus the NICAS in a large cohort of MCAS patients, aiming to identify patients presenting with MCAS who should undergo subsequent BM studies for the diagnosis of a primary or clonal MC disease. Our results demonstrated a higher accuracy and a greater rate of correctly classified patients for the REMA score versus the NICAS, particularly among men, patients presenting with anaphylaxis owing to any cause featuring urticaria and/or presyncope, and a blood-negative/BM-positive *KIT* mutational profile, which point to the need for more sensitive blood-based assays to detect *KIT*^{D816V}, when used alone. However, combined assessment of the REMAs and *KIT*^{D816V} mutational status in blood by ASO-qPCR might increase the diagnostic accuracy and thus the value of the REMAs in the diagnostic screening of MCAS patients with clonal MC disorders.

Acknowledgments

The authors thank their patients for their willingness to participate in this study. T.A. Rama and I. Alvarez-Twose conceptualized the study. T.A. Rama analyzed the data and wrote the initial draft of the manuscript. T.A. Rama, I. Torrado, E. Redondo, and A.Garbán. acquired clinical data. L. Sanchez-Muñoz, A.F. Henriques, M. Jara-Acevedo, P. Navarro-Navarro, C. Caldas, A. Mayado, J. Muñoz-González, A. García-Montero, and M. Mollejo acquired laboratory data. I. Alvarez-Twose, A. Moreira, and A. Órfão reviewed and edited the final version of the manuscript. All authors revised the manuscript critically for important intellectual content. All authors approved the final version of the manuscript.

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ONLINE REPOSITORY

Details of cases with modified classifications when serum baseline tryptase values were corrected by hereditary α-tryptasemia genotype

For the Spanish Network on Mastocytosis score (REMAs), a man with systemic mastocytosis (SM) and a REMAs of 2 who had been misclassified with a National Institutes of Health idiopathic clonal anaphylaxis score (NICAS) had a 18.2 μg/L serum baseline tryptase (sBT) value and a 3α2β *TPSAB1* genotype (one extra copy), which led to a corrected sBT value of 9.1 μg/L, and which led to a decreased REMAs of 1. Regarding the NICAS, patients whose score changed from above to below the cutoff of 2 or greater, once sBT levels were corrected by the hereditary α-tryptasemia genotype, included 1) a woman with SM who had 24.7 μg/L sBT and a 3α3β *TPSAB1* genotype (two extra copies), associated with Hymenoptera venom anaphylaxis presenting with syncope and a BM-positive/peripheral blood-negative *KIT* mutational profile, and a score of 3 with the NICAS, which decreased to 1 after sBT correction; 2) a woman with SM, with a 21.4 μg/L sBT value and a 2α3β *TPSAB1* genotype (one extra copy), whose score of 2 with the NICAS decreased to 0 after sBT correction to 10.7 μg/L; and 3) a woman with nonclonal mast cell activation syndrome misclassified with NICAS, who had 22.5 μg/L sBT levels and a 2α3β *TPSAB1* genotype (one extra copy), which decreased the NICAS to 1 after sBT values were corrected to 11.25 μg/L.

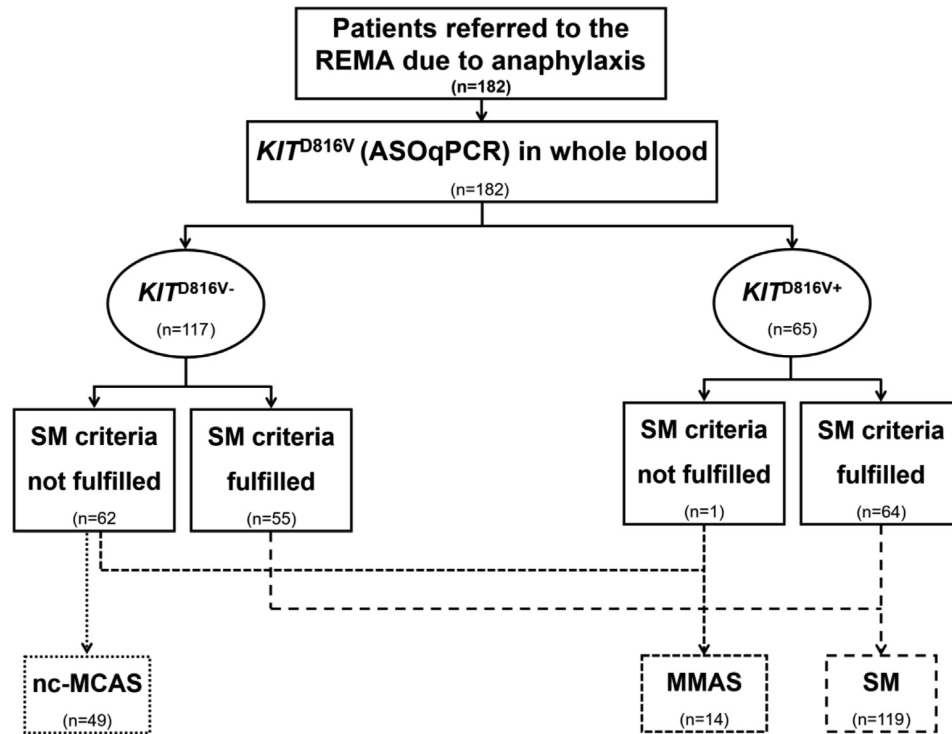

FIGURE E1. Flowchart illustrating how patients were evaluated during the prospective analysis performed in this study on the *KIT*^{D816V} mutational status in blood. *ASO-qPCR*, allele-specific oligonucleotide quantitative polymerase chain reaction; *BM*, bone marrow; *MMAS*, monoclonal mast cell activation syndrome; *nc-MCAS*, nonclonal mast cell activation syndrome; *REMA*, Red Española de Mastocitosis (Spanish Network on Mastocytosis); *SM*, systemic mastocytosis.

TABLE E1. Clinical, demographic, and laboratory features of MCAS patients grouped according to presence of HAT genotypes and presence of clonality

	Total			Nonclonal MCAS			Clonal MCAS		
Variable	**Non-HAT (n = 59)**	**HAT (n = 12)**	***P***	**Non-HAT (n = 28)**	**HAT (n = 5)**	***P***	**Non-HAT (n = 31)**	**HAT (n = 7)**	***P***
Men	30 (51%)	6 (50%)	1.0	14 (50%)	1 (20%)	.35	16 (52%)	5 (71%)	.43
Age, y	57 (24-81)	57 (32-69)	.93	59 (24-74)	60 (45-66)	.83	55 (33-81)	56 (32-69)	1.00
Triggers for anaphylaxis									
Hymenoptera	16 (27%)	6 (50%)	.12	6 (21%)	2 (40%)	.57	10 (32%)	4 (57%)	.39
Drugs	11 (19%)	2 (17%)	1.0	7 (25%)	2 (40%)	.6	4 (13%)	0 (0%)	1.00
Idiopathic	20 (34%)	2 (17%)	.32	9 (32%)	1 (20%)	1.00	11 (35%)	1 (14%)	.40
Food	7 (12%)	1 (8%)	1.00	3 (11%)	0 (0%)	1.00	4 (13%)	1 (14%)	1.00
Mixed	5 (8%)	1 (8%)	1.00	3 (11%)	0 (0%)	1.00	2 (6%)	1 (14%)	.47
Mucocutaneous symptoms	24 (41%)	6 (50%)	.55	18 (64%)	4 (80%)	.64	6 (19%)	2 (29%)	.62
Urticaria	18 (31%)	2 (17%)	0.33	15 (54%)	2 (40%)	1.00	3 (10%)	0 (0%)	.66
Pruritus	22 (37%)	2 (17%)	.17	16 (57%)	2 (40%)	.64	6 (19%)	0 (0%)	.57
Angioedema	9 (15%)	3 (25%)	.41	5 (18%)	1 (20%)	1.00	4 (13%)	2 (29%)	.30
Cardiovascular symptoms	50 (85%)	9 (75%)	.41	21 (75%)	2 (40%)	.15	29 (94%)	7 (100%)	1.00
Presyncope	40 (68%)	7 (58%)	.52	17 (61%)	1 (20%)	.15	23 (74%)	6 (86%)	1.00
Syncope (with or without presyncope)	37 (63%)	6 (50%)	.52	14 (50%)	1 (20%)	.35	23 (74%)	5 (71%)	1.00
Serum baseline tryptase, μg/L	15.3 (2.4-176)	26 (12-217)	.11	6.2 (2.4-51.8)	22.5 (12-39)	.11	23.3 (2.9-176)	27.3 (18.2-217)	.19
KIT mutations	31 (53%)	7 (58%)	.71	0 (0%)	0 (0%)	—	31 (100%)	7 (100%)	—
REMAs ≥2	39 (66%)	7 (58%)	.61	10 (36%)	1 (20%)	.64	29 (94%)	6 (86%)	.47
NICAS ≥2	38 (64%)	6 (50%)	.35	12 (43%)	1 (20%)	.63	26 (84%)	5 (71%)	.59

HAT, Hereditary α-tryptasemia; *MCAS*, mast cell activation syndrome; *NICAS*, National Institutes of Health Idiopathic Clonal Anaphylaxis Score; *REMAs*, Spanish Network on Mastocytosis score.

Results are expressed as numbers of patients from all patients in group and percentages in parentheses (rounded to units) or as median values and ranges in parentheses.

TABLE E2. Genetic features and sBT levels of patients with diagnosis of HAT distributed according to specific diagnostic subtypes of MCAS

Variable	Total (n = 71)	nc-MCAS (n = 33)	c-MCAS (n = 38)	*P*	MMAS (n = 4)	SM (n = 34)	*P*
TPSAB1 genotype							
0α4β	13 (18%)	10 (30%)	3 (8%)	**.03**	0 (0%)	3 (9%)	1.00
1α3β	23 (32%)	8 (24%)	15 (40%)	.21	0 (0%)	15 (44%)	.13
2α1β	2 (3%)	0 (0%)	2 (5%)	.5	1 (25%)	1 (3%)	.20
2α2β	21 (30%)	10 (30%)	11 (29%)	1.00	3 (75%)	8 (24%)	.65
2α3β	7 (10%)	4 (12%)	3 (8%)	.7	0 (0%)	3 (9%)	1.00
3α2β	2 (3%)	1 (3%)	1 (3%)	1.00	0 (0%)	1 (3%)	1.00
3α3β	2 (3%)	0 (0%)	2 (5%)	.5	0 (0%)	2 (6%)	1.00
3α4β	1 (1%)	0 (0%)	1 (3%)	1.00	0 (0%)	1 (3%)	1.00
Non-HAT patients	59 (83%)	28 (85%)	31 (82)%	.76	4 (100%)	27 (79%)	.57
sBT, μg/L	15.3 (2.4-176)*	6.2 (2.4-51.8)	23.3 (2.9-176)	**<.001**	15.1 (13.7-26.6)	26 (2.9-176)	.11
≤11.4	21 (36%)	18 (64%)	3 (10%)	**<.001**	0 (0%)	3 (11%)	.48
≥15	31 (53%)	6 (21%)	25 (81%)	**<.001**	2 (50%)	23 (85%)	.10
≥20	22 (37%)	3 (11%)	19 (61%)	**<.001**	1 (25%)	18 (67%)	.11
≥25	18 (31%)	3 (11%)	15 (48%)	**0.002**	1 (25%)	14 (52%)	.31
HAT patients	12 (17%)	5 (15%)	7 (18%)	.76	0 (0%)	7 (21%)	.57
sBT, μg/L	26 (12-217)*	22.5 (12-39)	27.3 (18.2-217)	.34	—	27.3 (18.2-217)	—
≤11.4	0 (0%)	0 (0%)	0 (0%)	1.00	0 (0%)	0 (0%)	1.00
≥15	11 (92%)	4 (80%)	7 (100%)	.22	0 (0%)	7 (100%)	**.003**
≥20	9 (75%)	3 (60%)	6 (86%)	.31	0 (0%)	6 (86%)	**.015**
≥25	6 (50%)	2 (40%)	4 (57%)	.56	0 (0%)	4 (57%)	.19

c-MCAS, Clonal mast cell activation syndrome; *HAT*, hereditary α-tryptasemia; *MMAS*, monoclonal mast cell activation syndrome; *nc-MCAS*, nonclonal mast cell activation syndrome; *sBT*, serum baseline tryptase; *SM*, systemic mastocytosis.

Results are expressed as numbers of patients from all patients in group and percentages in parentheses (rounded to units) or as median values and ranges in parentheses.

Statistically significant differences among groups are highlighted in bold.

*Statistically significant difference ($P = .011$).