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A distinct biomolecular profile identifies monoclonal mast cell disorders in patients with idiopathic anaphylaxis

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Abstract

Background—Clonal mast cell disorders are known to occur in a subset of patients with systemic reactions to Hymenoptera stings. This observation has prompted the question as to whether clonal mast cell disorders also occur in patients with idiopathic anaphylaxis (IA).

Objective—We sought to determine the prevalence of clonal mast cell disorders among patients with IA, criteria to identify those patients who require a bone marrow biopsy and whether the pathogenesis of IA involves a hyper-responsive mast cell compartment.

Methods—We prospectively enrolled patients with IA (≥3 episodes/yr) and who then underwent a medical evaluation that included a serum tryptase determination, allele-specific quantitative polymerase chain reaction (ASqPCR) for *KIT* D816V and a bone marrow examination. Mast cells were cultured from peripheral blood CD34+ cells and examined for releasibility following FcεRI aggregation.

Results—Clonal mast cell disease was diagnosed in 14% of patients referred with IA. ASqPCR for the *KIT* D816V mutation was a useful adjunct in helping identify those with systemic mastocytosis (SM) but not monoclonal mast cell activation syndrome (MMAS). A modified overall clonal prediction model was developed using clinical findings, a serum tryptase

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determination and ASqPCR. There was no evidence of a hyper-responsive mast cell phenotype in patients with IA.

Conclusion—Patients with clonal mast cell disease may present as idiopathic anaphylaxis. Distinct clinical and laboratory features may be used to select those patients more likely to have an underlying clonal mast cell disorder (MMAS or SM) and thus candidates for a bone marrow biopsy.

Keywords

Anaphylaxis; Mast cells; Mastocytosis; Monoclonal mast cell activation syndrome; Mast cell activation; Tryptase; KIT; Allele-specific quantitative PCR

INTRODUCTION

Idiopathic anaphylaxis (IA) is a diagnosis of exclusion and is based on the inability to identify a cause and effect relationship for a given anaphylactic event. Once the diagnosis is made, an associated question is whether there is an underlying clonal disorder such as systemic mastocytosis (SM). Patients with a diagnosis of SM are reported to have an increased prevalence of anaphylaxis[1], both unexplained and associated with hymenoptera sensitivity.[2–4] While it is also known that some patients with IA have an underlying clonal mast cell disease,[5] the frequency of the association between IA and clonal mast cell disease and how to identify patients with IA who should undergo a marrow examination to confirm this diagnosis has not been thoroughly explored.

Two studies have attempted to define characteristics that identify patients with severe mast cell mediator related disease that are likely to have a clonal mast cell disorder.[4, 6] The first study examined patients presenting with severe mast cell mediator related symptoms and compared the characteristics of this group to patients with a known diagnosis of indolent SM (ISM) to identify clinical and biological characteristics that would predict those patients who would be more likely to have a clonal mast cell disorder and thus need a marrow examination.[6] A second study retrospectively examined records of patients with clinically suspected clonal mast cell disease based on one or more episodes of unexplained anaphylaxis and who had a bone marrow examination. Based on their observations, they then suggested modifications to the previous scoring system.[4]

These reports led us to design a prospective study to determine the prevalence of a clonal mast cell disorder in patients experiencing frequent episodes of IA without clinical evidence of mastocytosis such as maculopapular cutaneous mastocytosis. Patients underwent a clinical evaluation, which included the analysis for *KIT* D816V and a bone marrow biopsy. As an additional objective, we examined the bone marrow mast cell compartment in vivo and mast cells cultured from peripheral blood to determine whether there was evidence of a hyperresponsive mast cell phenotype in patients with IA. As will be shown, approximately one in seven patients with IA had a clonal mast cell disorder. We found no evidence of a hyperresponsive mast cell phenotype. We also present a modified scoring system with increased specificity and sensitivity for identification of patients with recurrent IA who are candidates for a bone marrow biopsy.

METHODS

Subjects

Fifty-six subjects (age 13–69 years) with a diagnosis of IA defined by current guidelines[7, 8] and meeting protocol entry requirements (supplemental Table E1) were enrolled from 22 states, and 1 Canadian province (supplemental Figure E1) over a study period of 6 years on an IRB-approved NIH protocol (NCT00719719) for further evaluation. Anaphylaxis was diagnosed by the referral physician using criteria from the summary report on anaphylaxis[9] Patients had to experience ≥3 episodes of unexplained anaphylaxis within 12 months of study entry, at least one episode in the past four months and at least one event evaluated in a medical facility in proximity to the episode and where the diagnosis of anaphylaxis was confirmed by documenting hypotension and other physical findings.[9] The median age of patients enrolled was 43 years. Thirty-seven (66.1%) where female and 19 (33.9%) were male. The majority of patients were Caucasian (93%). Upon enrollment to the NIH protocol, all patients underwent a complete physical examination, with serum IgE and baseline serum tryptase (bST) measured and a bone marrow aspirate and biopsy performed. Healthy volunteers (HV) for mast cell comparison studies were enrolled in an IRB-approved protocol (NCT00806364). All subjects provided informed consent prior to enrollment.

Laboratory Studies

Serum tryptase, IgE values and venom-specific IgE—bST levels were determined using a fluoroenzyme Immunoassay (Phadia Immuno CAP, Uppsala, Sweden) at CLIA-approved labs. The normal reference range for this assay is 0.00 – 11.50 ng/ml. The serum IgE level was determined using the Immulite XPI, solid phase chemiluminesence assay (Siemens Medical Solutions, Malvern, PA). IgE levels to honey bee and yellow jacket were screened using a flouroenzyme immunoassay (Phadia Immuno CAP) with a detection range of 0.35 to >100 IU/ml in Dr. Platts-Mills' laboratory, where values greater than 0.35 IU/ml are considered positive.

Allele-specific quantitative PCR (ASqPCR) on peripheral blood—Genomic DNA (gDNA) was prepared from 200 uL of blood collected in EDTA from 37 patients and extracted using a QIAamp DNA blood mini kit (QIAgen, Hilden, Germany) in a volume of 100 ul of elution buffer. Genomic DNA from HMC1.2 cells[10] was used as the KIT D816V mutation positive control. Genomic DNA from peripheral blood of a HV was used as a negative control. The concentration of each DNA sample was determined using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Both mutation-specific and control real-time qPCR assays for *KIT* D816V were performed for each sample in the same plate using the TaqMan Universal PCR Master Mix with AmpErase UNG on the 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) as described.[11] Each real-time qPCR reaction was performed in triplicate with 50 ng of gDNA in a total volume of 25 ul. Results were analyzed using SDS software version 1.3.1 (Applied Biosystems, Grand Island, NY). Samples with two of three or three of three analyses generating a threshold cycle (Ct) value below 41 were defined as mutation positive. Mutation negative samples tested negative with zero of three reactions producing a Ct value

below 44.[12] The percentage of the cells carrying the KIT D816V allele among the total number of nucleated blood cells was then calculated as described.[11]

Bone marrow examination

Bone marrow trephine biopsies were fixed in B-5 fixative, embedded in paraffin and processed for morphology using standard procedures.[13] Immunohistochemical studies with anti-tryptase, anti-CD117 (Cell Marque, Hot Springs, AR) and anti-CD25 antibodies (Vision BioSystems, Norwell, MA) were performed using immunoperoxidase staining and an automated immunostainer (Ventana Medical System, Tucson, AZ) per manufacturer's instructions. Images were obtained using an Olympus DP72 digital camera (BD Canto II, San Jose, CA). Bone marrow biopsies were inspected and scored in a blinded fashion by a single pathologist (I.M.). Multi-parameter flow cytometry was performed after bone marrow aspirates were processed and stained with antibodies for CD2, CD25, CD45 and CD117 as described.[13] Activation markers were identified with additional antibodies for CD11c, CD35, CD59, CD63, CD69 and CD203c. The DIVA and FCS Express (De Novo software, Glendale, CA) programs were used for data analysis.[13] Bone marrow mononuclear cells were obtained from the Ficoll gradient in PBS. The CD25+ cells were magnetically labeled with CD25 Microbeads II (Miltenyi Biotec, San Diego, CA), then the cell suspension was loaded onto a MACS® column and placed in the magnetic field of the MACS separator. The labeled CD25+ cells were retained on the column and eluted as the positively selected cell fraction. The detection of the KIT D816V mutation was performed by RT-PCR/RFLP. The targeted gene region was amplified and the PCR products subjected to restriction enzymes in order to obtain fragments for analysis as described.[13]

Mast cell culture and activation

Peripheral Blood-Derived CD34+ Mast Cells (PBDMCs) were cultured from 100 ml of blood obtained from subjects and HV as described.[14] Briefly, mononuclear cells were separated by density gradient centrifugation, and a human progenitor cell enrichment kit was used to obtain CD34+ cells by negative selection as per the manufacturer’s guidelines (Stemcell Technologies, Vancouver, Canada). Cells were then cultured in Stempro-34 Media (Life Technologies, Grand Island, NY) with 100 ng/ml rSCF and 100 ng/ml rIL6 (PeproTech, Rocky Hill, NJ) for 7 weeks, with 30 ng/ml rIL3 added during the first week only.[14] The number of cells in culture was determined at 6 weeks and normalized to percent CD34+ cells measured at the start of culture. At 6–7 weeks, PBDMCs were sensitized overnight in media containing biotinylated-anti-IgE (100ng/ml). After sensitization, the cells were activated by crosslinking with streptavidin (SA) (Sigma-Aldrich, St. Louis, MO) and percent release of β-hexosaminidase calculated.[15]

Statistics

bST values were compared using student t test. A Wilcoxon Rank Sum test was used to compare absolute bone marrow mast cell values between different disease categories and controls. The Mann-Whitney non-parametric analysis was use to compare surface antigens between IA, monoclonal mast cell activation, (MMAS), ISM and HV groups. Scatterplots illustrate relationships between disease categories, tryptase, bone marrow mast cells and IgE.

Corresponding Spearman rank correlation coefficients were computed. Statistical graphics and analysis were performed using PRISM 6th ed (GraphPad, La Jolla, CA).

RESULTS

Diagnosis of clonal mast cell disease

All fifty-six patients underwent an evaluation including a bone marrow examination with flow cytometry and KIT mutational analysis to determine the presence or absence of an underlying clonal mast cell disorder. Four patients were diagnosed with ISM and four with MMAS based on existing criteria (Table 1)[16, 17] leaving 48 patients with IA who had no evidence of a clonal disorder. Marrow findings in Figure 1 illustrate the spectra of marrow findings in patients referred with the diagnosis of IA. Figure 1A shows results of a representative marrow biopsy and flow cytometric analysis for CD25 from a patient with IA who did not meet the criteria for a clonal mast cell disorder. All marrow mast cells are CD25 negative and marrow KIT D816V mutation analysis is negative. The patient did have an elevated bST (29.4 ng/ml). In Figure 1B, findings are shown for a patient diagnosed with MMAS (bST 16.7 ng/ml). This patient's marrow exhibited both CD25+ and CD25- mast cell populations and was positive for the D816V mutation in the enriched CD25+ mononuclear cell population. The patient whose marrow results are shown in Figure 1C had a bST of 88.7 ng/mL and met both major and minor criteria for the diagnosis of ISM.

Of interest, results of peripheral blood analysis for the presence of *KIT* D816V were consistently negative in patients diagnosed with IA alone [100%] and positive in patients with ISM (allelic burden ranged from 0.037–1.19%), who showed mast cell aggregates of over 15 mast cells on the bone marrow biopsy [100%]. However, PB ASqPCR was negative in all patients with MMAS and in one patient with ISM who had no marrow aggregates, the major criterion for the diagnosis of SM. Thus, based on this test alone, the negative and positive predictive values for ASqPCR to diagnose ISM are 90.74% and 100% respectively, in the population of patients with IA.

As shown in Table 2, patients with IA (no evidence of a clonal mast cell disorder) were more likely to be female but age did not differ amongst the three groups. IgE levels were lowest in patients with ISM, but the median values were not significantly different between groups. The bST values were significantly higher in patients with MMAS and ISM compared to patients with IA ($p<0.0001$). Patients with IA, on average, had more frequent episodes of anaphylaxis per year and a longer duration of disease compared to patients with clonal disorders. Only one patient with IA and none with clonal disease reported clinical symptoms to hymenoptera. Thus, venom sensitization was not a distinguishing feature of clonal mast cell disease in this population.

Examination of the mast cell compartment in vivo and in vitro

We next examined the characteristics of the bone marrow mast cell compartment in patients with IA, MMAS and ISM versus findings in HV. The absolute mast cell count was similar between patients with IA and HV, while the mast cell count was greater in patients with MMAS and ISM (Table 3). In addition, CD25, CD2, CD11c, CD 59, CD 69 and CD35 MFI

ratios, as expected, were elevated in patients with ISM,[18] but not in patients with IA or MMAS. We, as expected, found over-expression of CD63 on mast cells in the marrow of patients with MMAS and ISM and CD203C on mast cells in patients with ISM, but not in patients with IA and HV (Figure 2 A, B). Thus, an assessment using immunophenotypic markers of mast cells in marrow provided no evidence that the mast cell compartment in patients with IA differed from what was observed in HV. We were unable to obtain sufficient mast cells from the marrow aspirates of patients with IA or from HV in order to examine their activation through the IgE and C5a receptors, as has been reported in a study on bone marrow mast cells from patients with clonal disease.[19]

To further examine the mast cell compartment, mast cells were cultured from 48 patients with IA, four with MMAS, and four with ISM. Each culture was followed for mast cell growth and at 6–7 weeks, IgE-mediated degranulation was determined in comparison to a mast cell culture from a HV performed in parallel. Mast cell growth from patients with MMAS and HV was similar, while it was statistically greater in patients with IA and ISM (Fig. 2C). IgE-mediated mast cell degranulation, as assessed by B-hexosaminidase release, was similar among all groups (Figure 2D). Hence, we found no evidence that the mast cells cultured from patients with IA were intrinsically more reactive.

Tryptase determinations in association with anaphylaxis and over time

We were able to obtain serum tryptase levels in 13 patients at baseline and within 4 hours after an episode of anaphylaxis. All patients had a significant rise in serum tryptase[17] associated with an anaphylactic episode (Figures 3A, B). Patients with a clonal disorder when compare to IA alone had a greater increase in serum tryptase post event (average 502 vs 25 ng/ml). We obtained sequential bST levels in 35 patients. The tryptase levels in patients with IA tended to remain stable, while patients with clonal mast cell disorders trended upward (Fig 3C, D)).

Predictors of clonal mast cell disorders

Two groups have proposed scoring systems termed either the REMA score or the Modified REMA score to predict the likelihood of a clonal mast cell disorder based on clinical findings exclusive of a bone marrow biopsy.[4, 6] When either of these approaches were applied to our patient population, the probable diagnosis of a clonal disorder then verified by a bone marrow examination had a sensitivity and specificity of 62.5% and 72.9%, respectively with either system. We created a modified version of the clinical variables, changed the serum tryptase limits from 25 and 20 ng/ml, respectively to ≤ 11.4 ng/ml for IA and > 11.4 ng/ml for clonal disease and added results of ASqPCR (Table IV A). When applied to the 37 patients from our cohort who had ASqPCR performed with a score of ≥2, the diagnosis of a clonal mast cell disorder was obtained with a sensitivity and specificity of 75% and 100%, respectively (Table IV B). Thus, using this further modified scoring system termed the NIH Idiopathic Clonal Anaphylaxis Score (NICAS), no patients with IA would have undergone an unnecessary bone marrow procedure, and all patients with ISM would have been diagnosed correctly, as well as 75% of patients with MMAS.

DISCUSSION

Here, we report the first prospective study to investigate the prevalence of a clonal mast cell disorder in patients with frequent episodes of IA. Patients with IA entered into this study were referred with this diagnosis by physicians who found no precipitating factors related to anaphylaxis and no reason to suspect a clonal mast cell disorder including the presence of cutaneous mastocytosis. Upon admission to the NIH Clinical Center, all 56 patients had an evaluation, which included a bone marrow biopsy and aspirate. Based on the results of a marrow biopsy and aspirate, four patients were diagnosed with ISM (7%) using WHO guidelines[16] and four patients with MMAS (7%)[20–22]. Note that because not all patients diagnosed with IA were able to have tryptase levels drawn in association with their episodes of anaphylaxis, as the concept of MCAS has evolved and now requires demonstration of a rise in mast cell mediators, some would now use the term monoclonal mast cells of uncertain significance (MMUS) in referring to these four patients.[23] The extent of bone marrow pathology in patients that fulfilled criteria for ISM was consistent with the relatively low sBT values. The bone marrow pathology in patients with MMAS fulfilled only one to two minor criteria for ISM consistent with the consensus diagnosis of MMAS (E2-A).[22] As an example, one patient with MMAS had two populations of mast cells (CD25+ and CD25−) on flow cytometry indicating the presence of both normal and clonal mast cells (Fig. 1 A–B). This highlights the importance of performing a bone marrow analysis, which examines all parameters listed in the WHO criteria for the diagnosis of mastocytosis (Tables E2 A, B).

The flow cytometric analysis showed that immunophenotypic characteristics of bone marrow mast cells were abnormal in patients with ISM or MMAS (Table III, Figure 2 A, B) with expression of surface makers such as CD25 and up-regulation of CD63 and CD203c. The bone marrow mast cell compartment in patients with IA was no different from that of HV including the degree of in vivo activation as shown by expression of CD63 and CD203c. Thus, there was no evidence of in vivo activation of mast cells in the bone marrow of patients with IA.

CD34+ cells from the peripheral blood of patients with ISM or IA when cultured produced more mast cells compared to those obtained from HV. When all groups were compared, there was no difference in mast cell responsiveness as examined by IgE-dependent beta-hexosaminidase release (Figure 2 C, D). Thus, we found no evidence that mast cells from patients with IA were hyperresponsive to IgE-mediated activation when compared to mast cells from HV, although their growth appeared greater for reasons, which are unclear.

The symptoms reported in patients with IA were typical of those associated with mast cell mediator release. Most patients (~91%) had cutaneous manifestations associated with episodes of anaphylaxis. Other signs and symptoms included hypotension, wheezing, shortness of breath, laryngospasm, vomiting and diarrhea.[6]

Findings that supported the diagnosis of a clonal disorder included relatively higher bST values and more severe reactions. Only one patient with IA had a history of a clinical reaction to venom with supporting laboratory tests. None of the patients with clonal disease reported an adverse clinical response to venom stings although two were positive for serum

specific-IgE (Table II). Hence, venom-induced anaphylaxis was not predictive of clonal disease.

Our study, which focused on patients with severe recurrent IA, differs from a recent study that reported the presence of mastocytosis in patients presenting with any cause of anaphylaxis including venom and regardless of a previous diagnosis of ISM. They found an incidence of clonal disease of 4.3%, compared to 14% in our study. In their study, 75% had a clinical reaction to wasp, also differing from our cohort. However, their study also recognized the value of ASqPCR, which we similarly found useful in identifying clonal disorders.[24–27] We would note that in our study patients with the diagnosis of IA who had a marrow performed by their physician and which was positive for mastocytosis would not have been referred to our study, so the prevalence of a clonal mast cell disorder in patients with IA is likely to be greater that 14%.

Another study, as previously mentioned, resulted in a scoring system (REMA score) designed to predict clonality among patients presenting with severe mast cell mediator related symptoms.[6] This scoring system was further refined in a subsequent study previously discussed and which retrospectively examined records of patients with clinically suspected clonal mast cell disease based on one or more episodes of unexplained anaphylaxis and who had a bone marrow examination.[4] When either of these algorithms was applied to our patient population, the positive and negative predictive values for clonality were 27.8% and 92.1 %, respectively (Table IV). We further modified these scoring systems for our population with IA using a cut-off of the serum tryptase level at 11.4 ng/ml and ASqPCR to detect the presence or absence of *KIT* D816V in peripheral blood that increased the positive and negative predictive values to 100% and 96%, respectively. Note, however, that our algorithm was specifically developed for the evaluation of patients presenting with severe recurrent IA and thus is not meant to replace previous scoring systems (Table IV). These findings and recommendations are in line with previous guidelines, including the REMA score and the ECNM guidelines.[6, 17]

Serum tryptase measurements in association with an episode of anaphylaxis has been shown to be valuable in implicating the mast cell compartment in anaphylaxis.[28] We found the rise in serum tryptase with an anaphylactic event was higher in patients with a clonal disease as compared to non-clonal disease (Fig. 3 A, B). In addition, when followed over time, most patients (83.3%) with clonal disease demonstrated a gradual increase in bST as compared to patients without clonal disease (7.1%) (Figure 3 C, D). Since the management for ISM is generally symptomatic, these parameters could be used as additional measures to follow patients with mast cell mediator symptoms for a more tempered diagnostic approach.

In this prospective study of IA, we diagnosed 14% with a clonal mast cell disorder. None of these patients presented with venom anaphylaxis and therefore this patient group differed from those described by other groups.[2, 29] Although no single parameter was diagnostic for a clonal disorder, peripheral blood ASqPCR to identify the presence of *KIT* D816V was beneficial in identifying those patients with IA and a clonal disorder with a 100% positive predictive value. However, the sensitivity of ASqPCR alone for clonal disease was only 37.5%. Our scoring system reliably screens out patients without clonal disease who would

otherwise undergo an unnecessary bone marrow procedure. This “NIH Idiopathic Clonal Anaphylaxis Score” (NICAS) utilizing ASqPCR, increased the sensitivity and specificity of predicting a clonal disorder in patients with IA to 75% and 100%, respectively. We offer this study and its conclusions as a contribution to the identification of patients with IA who have an underlying clonal mast cell disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ASqPCR	Allele-specific quantitative polymerase chain reaction
bST	Baseline serum tryptase
HV	Healthy volunteers
HMC	Human mast cells
IA	Idiopathic anaphylaxis
ISM	Indolent systemic mastocytosis
MFI	Mean fluorescence intensity
MMAS	Monoclonal mast cell activation syndrome
MMUS	Monoclonal mast cells of uncertain significance
PBDMC	Peripheral Blood-Derived CD34+ Mast Cell
REMA	Spanish Network on Mastocytosis
SCF	Stem cell factor
SM	Systemic mastocytosis
WHO	World Health Organization

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Key Messages

- Fourteen percent of patients referred with the diagnosis of IA had an underlying clonal mast cell disorder.
- A distinct biomolecular profile identifies those patients with IA who are likely to have a clonal mast cell disorder and thus candidates for a bone marrow study to confirm the diagnosis.
- We did not find evidence of a hyperresponsive mast cell phenotype in patients with IA in the absence of a clonal mast cell disorder.

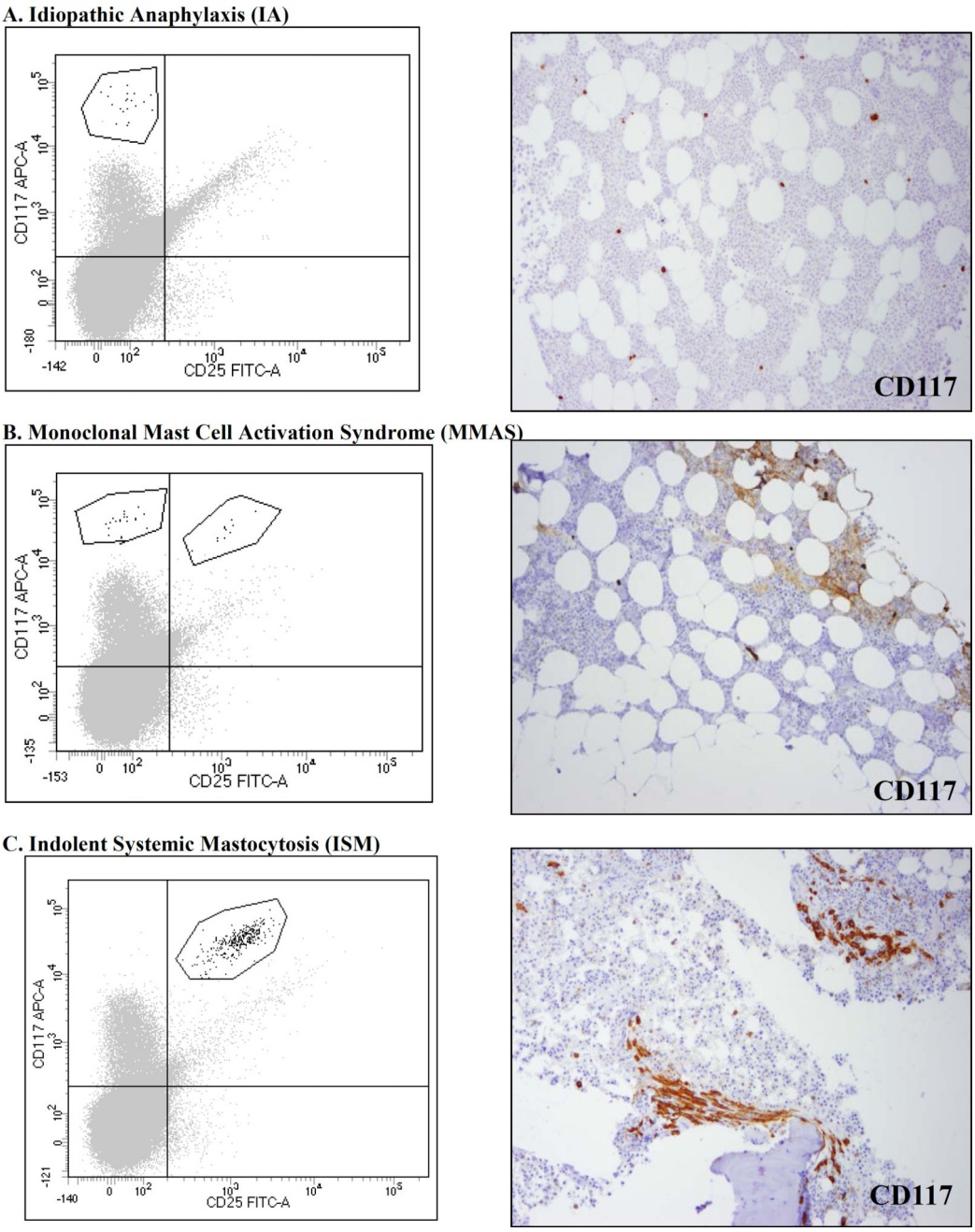

Figure 1. Bone marrow mast cell flow cytometry and immunohistochemistry in patients with IA (A), MMAS (B), ISM (C)

Flow cytometric results show that CD117+ mast cells gain CD25 surface expression in clonal disease, illustrated by no abnormal CD25+ mast cells in IA (A), two populations of mast cells in MMAS (CD25+ and CD25−, B) and abnormal CD25+ mast cells in ISM (C). Bone marrow biopsies showed no mast cell clusters in IA and MMAS with multiple clusters in ISM.

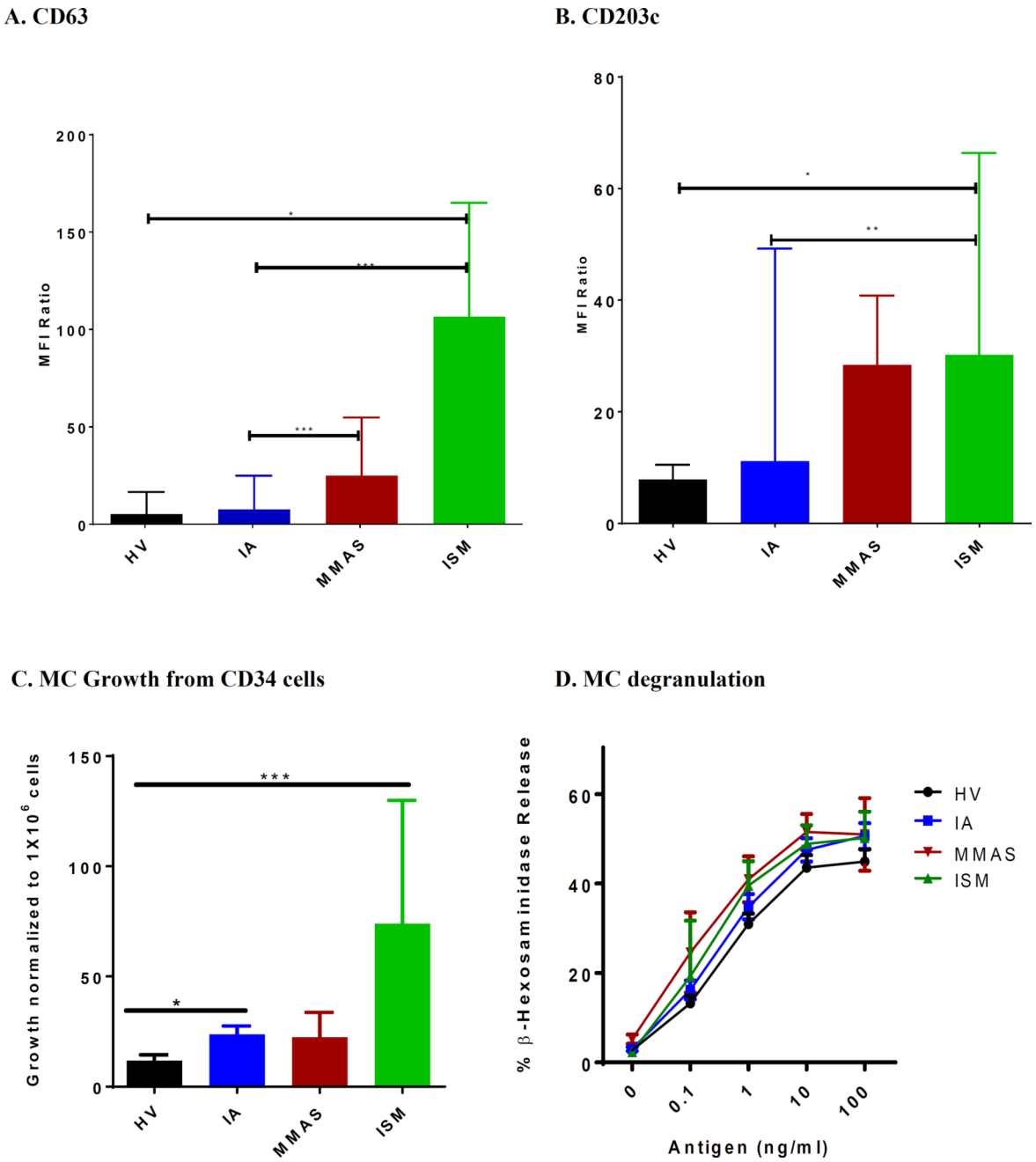

Figure 2. Immunophenotypic mast cell markers expressed by bone marrow mast cells, mast cell growth and degranulation of cultured human mast cells

Mast cell markers, CD63 and CD203c, are compared in non-clonal (HV, IA) and clonal patient populations (MMAS, ISM) (A, B). Patients with ISM demonstrated the highest expression of CD63 and CD203c, which were significantly higher than both non-clonal populations. Non-clonal populations were similar. Mast cell proliferation from CD34 positive progenitor cells produced more mast cells from patients with IA or ISM (C). Degranulation of mast cells through the IgE receptor was similar amongst groups. (D) (A-*p=0.02, ***p<0.0001), (B-*p=0.04, **p=0.002), (C-*p=0.01, ***p=0.0002)

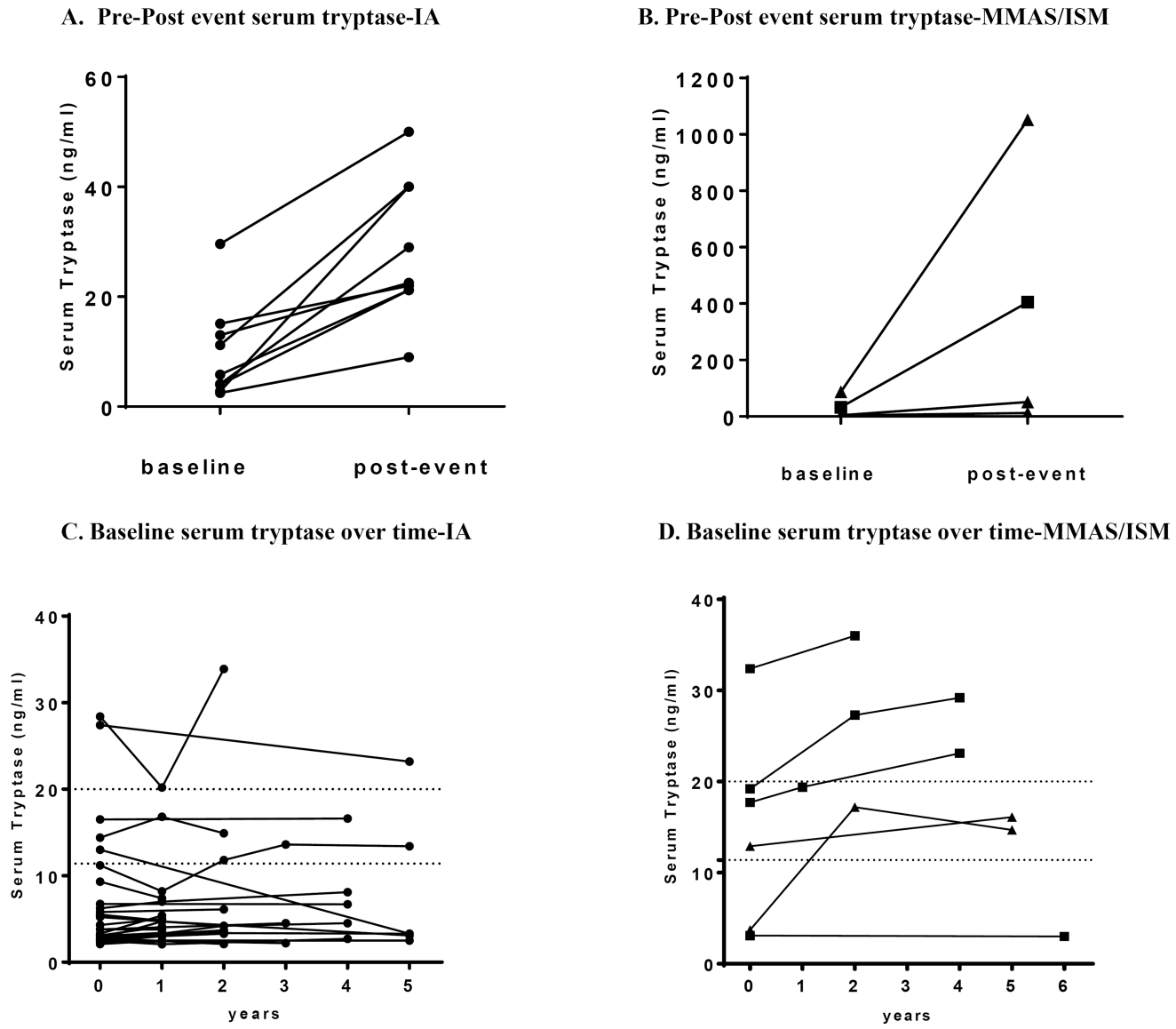

Figure 3. Pre-post event serum tryptase (A, B) and baseline serum tryptase over time (C, D) in non-clonal and clonal mast cell disease

When compared to baseline, both patients with IA (p=0.001) (A) and with clonal disease (MMAS, ISM) (p=0.002) (B) had a significant rise in serum tryptase with an anaphylactic event. However, the average increase was 502 vs 25 ng/ml for clonal vs IA, respectively. In most patients with IA, the baseline serum tryptase remained stable over time (C), while the majority of patients with clonal disease experienced a gradual rise over time (D). IA (Circles), ISM (triangles), MMAS (squares)

Table I

Final diagnosis among patients presenting with IA

Diagnosis (N)	MC aggregates >15 MCs	Spindle-shaped >25% MCs	CD25+ MCs	sBTryptase >20 ng/ml	BM *KIT* D816V	Blood *KIT* D816V#
IA (48)	0/48	0/48	0/48	1/48	0/48	0/29
MMAS*(4)	0/4	2/4	1/4	2/4	3/4	0/4
ISM (4)	3/4	4/4	4/4	2/4	4/4	3/4

* Two positive for BM D816V in CD25-enriched cells only. MC-mast cell, BM-bone marrow.

sBTryptase-serum baseline tryptase.

Blood *KIT* D816V-allele-specific PCR, # Performed on 37 patients.

Table II

Patient characteristics

Final Diagnosis	M/F	Age median (range)	Serum IgE IU/ml median (range)	sBTryptase ng/ml median (range)	Anaphylaxis frequency number/yr avg. (range)	Anaphylaxis duration Yrs avg. (range)	Venom Sensitivity by Clinical Hx*
IA	14/34	43.0 (13–69)	97.5 (5.1–2457)	4.1 (1.2–29.6)	9 (3–24)	7 (1–34)	1/48
MMAS	3/1	48.5 (40–53)	87.6 (31.8–128)	18.4 (3.1–32.4)	6 (5–8)	4 (1–7)	0/4
ISM	2/2	35.5 (17–58)	40.8 (21.2–805)	18.6 (3.7–88.7)	5 (3–9)	3 (1–8)	0/4

sBTryptase-serum baseline tryptase. P values for serum sBT for MMAS and ISM compared to IA are 0.0001 for both.

*All patients underwent ImmunoCap for IgE to honeybee and yellow jacket venoms. Seven of 48 with IA, 1 of 4 with MMAS and 1 of 4 with MMAS were positive.

Table III

Immunophenotypic characteristics of bone marrow mast cells

Diagnosis	Mast Cell Abs Count†	CD25 MFI Ratio (range)	CD2 MFI Ratio (range)	CD11c MFI Ratio (range)	CD35 MFI Ratio (range)	CD59 MFI Ratio (range)	CD63 MFI Ratio (range)	CD69 MFI Ratio (range)	CD203c MFI Ratio (range)
IA	**10.50** (4–65) n= 44	**1.02** (0.05–2.2) n= 44	**0.79** (0.08–3.7) n=43	**5.09** (0.35–25.3) n= 39	**1.14** (0.02–14) n= 42	**29.09** (1.71–70.8) n= 42	**7.54** (0.21–24.9) n= 41	**2.63** (0.0–10.7) n= 40	**11.13** (0.40–49.2) n= 40
MMAS*	**25.50** (16–142) n= 4	**1.26** (0.53–7.8) n= 4	**1.16** (0.61–137.8) n= 4	**5.45** (2.46–11.3) n= 3	**0.98** (0.57–2.5) n= 3	**35.78** (32.47–128.3) n= 3	**24.90** (18.40–54.7) n= 3	**2.75** (1.5–3.6) n= 3	**28.41** (15.98–40.8) n= 2
ISM	**216.0** (69–327) n= 4	**4.79** (2.68–6.9) n= 4	**14.87** (6.47–39.3) n= 4	**12.35** (5.38–31.3) n= 4	**15.47** (4.75–35.4) n= 4	**85.86** (64.62–134.6) n= 4	**106.60** (17.30–165) n= 4	**5.99** (5.13–8.9) n= 4	**30.20** (15.84–66.4) n= 4
HV	**10.0** (7–36) n= 4	**0.94** (0.8–1.3) n= 4	**0.67** (0.5–1.2) n= 4	**4.40** (4.2–13.2) n= 4	**1.08** (0.5–1.4) n= 4	**33.50** (24.9–56) n=4	**5.10** (3.4–16.5) n= 4	**2.40** (1.6–4.2) n= 4	**7.89** (5.15–10.5) n= 4

*1 patient with split mast cell population in MMAS category excluded from analysis for the following surface antigen marker ratios: CD11c, CD35, CD59, CD63, CD69 and CD203c. There was insufficient sample to complete labeling for all markers. Data expressed as a median (range). MFI=mean fluorescence intensity.

†Number of mast cells per 300,000 cells counted by flow on a marrow aspirate.

Table IV

Clonal mast cell predictability tables

A. Mast cell activation symptom scores-NICAS		
VARIABLE		**SCORE**
GENDER	Male	+1
	Female	−1
CLINICAL SYMPTOMS	Absence of angioedema	+1
	Flushing	−1
	Urticaria	+1
	Syncope	+3
TRYPTASE	<11.4 ng/ml	−1
	>11.4 ng/ml	+1
ALLELE-SPECIFIC PCR	Negative	−1
	Positive	+3

B. Sensitivity and Specificity for clonal prediction scores

Scoring System on NIH patient data	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
REMA score[6]	62.5% (24.4–91.5%)	72.92% (58.1%–83.4%)	27.78% (9.7%–53.5%)	92.11% (78.6%–98.3%)
Modified REMA score[7]	62.5% (24.4–91.5%)	72.92% (58.1%–83.4%)	27.78% (9.7%–53.5%)	92.11% (78.6%–98.3%)
ASqPCR	37.5%	100%	100%	90.57%
NICAS with ASqPCR	75% (34.9%–96.8%)	100% (92.6%–100%)	100% (54.1%–100%)	96% (86.3–99.5%)

NICAS-NIH Idiopathic Clonal Anaphylaxis Score. Total score ≥2 is predictive of clonal disease.