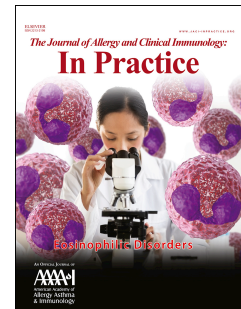


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# Diagnosis of primary mast cell disorders in anaphylaxis: value of KIT D816V in peripheral blood

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## Abstract

### *Background*

Anaphylaxis is frequent in patients suffering from primary mast cell disorders (PMCD). In patients without mastocytosis in the skin (MIS) and a baseline serum tryptase (bST) < 30 ng/mL, the diagnosis of PMCD is challenging. In these patients, detection of the KIT D816V mutation in peripheral blood (PB) has been suggested as screening tool for a PMCD.

### *Objective*

In this study we investigated whether KIT D816V in PB can contribute to the decision to perform a bone marrow (BM) biopsy in patients with anaphylaxis without MIS and a bST < 30 ng/mL.

### *Methods*

We selected 74 patients with severe anaphylaxis without MIS and a bST < 30 ng/mL. All underwent a BM biopsy. KIT D816V mutation was quantified in both PB and in BM using digital droplet polymerase chain reaction (ddPCR).

### *Results*

Diagnosis of a PMCD was established in 40 patients (54%). Median bST for patients with and without PMCD was respectively 9.5 (4.2-27) ng/mL and 4.9 (2.2-20.3) ng/mL ( $P < .001$ ). KIT D816V in PB was detected in 16 out of 40 (40%) patients with PMCD. KIT D816V in BM was detected in 22 out of 40 (55%) patients with PMCD.

### *Conclusion*

In patients without MIS and a baseline serum tryptase < 30 ng/mL who experience anaphylaxis, determination of KIT D816V mutation in PB is of limited help in deciding when to proceed to a BM biopsy. Therefore, KIT D816V in PB mutation analysis should be interpreted together with scoring tools to make a better assessment in identifying patients who should undergo BM-biopsy.

### *Clinical trial registration number:*

B300201836890

**Highlight box**

*What is already known about this topic?*

Detection of a KIT D816V mutation in extracutaneous cells, is a minor criterium for the diagnosis of systemic mastocytosis. Recently, several techniques have been introduced for its' detection in peripheral blood.

*What does this article add to our knowledge?*

Sixty percent of patients with anaphylaxis, without mastocytosis in the skin, a low mast cell burden and a negative KIT D816V in peripheral blood, have a primary mast cell disorder after a bone marrow biopsy.

*How does this study impact current management guidelines?*

In patients with low mast cell burden, KIT D816V in PB mutation analysis should be interpreted together with scoring tools to make a better assessment in identifying patients who should undergo bone marrow biopsy.

**Key words:**

Primary mast cell disorder, monoclonal mast cell activation syndrome, mastocytosis, KIT D816V mutation, anaphylaxis, mastocytosis in the skin, tryptase, mast cell

## 91 Abbreviations

|              |                                                                    |
|--------------|--------------------------------------------------------------------|
| AAAAI        | American Academy of Allergy Asthma and Immunology                  |
| BM           | Bone marrow                                                        |
| bST          | baseline serum tryptase                                            |
| CD           | Cluster of differentiation                                         |
| ddPCR        | digital droplet polymerase chain reaction                          |
| EAACI        | European Academy of Allergy and Clinical Immunology                |
| ECNM         | European Competence Network on Mastocytosis                        |
| H $\alpha$ T | hereditary alpha-tryptasemia                                       |
| ISM          | Indolent systemic mastocytosis                                     |
| ISM-AHN      | Indolent systemic mastocytosis with associated neoplastic neoplasm |
| MC           | Mast cell                                                          |
| MIS          | Mastocytosis in the skin                                           |
| MMAS         | Monoclonal mast cell activation syndrome                           |
| mREMA        | Modified REMA score                                                |
| NICAS        | NIH idiopathic clonal anaphylaxis score                            |
| NIH          | National Institute of Health                                       |
| PB           | Peripheral blood                                                   |
| pdSM         | pre-diagnostic systemic mastocytosis                               |
| PMCD         | primary mast cell disorder                                         |
| REMA         | Red Española de Mastocitosis (Spanish Mastocytosis Network)        |
| SM           | Systemic mastocytosis                                              |
| SSMC         | population of $\geq 25\%$ spindle shaped mast cells                |
| VIT          | Venom immunotherapy                                                |
| WHO          | World Health Organization                                          |

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## Introduction

Anaphylaxis is a potentially life-threatening, systemic reaction and an important reason for referral to an Allergology/Immunology outpatient clinic<sup>1</sup>. The prevalence of anaphylaxis is estimated to be increased by a 1,000-fold in adults with an underlying primary mast cell disorder (PMCD) as compared to patients without PMCD<sup>2</sup>. These patients generally also experience more severe grades of anaphylaxis than patients without underlying PMCD. Hymenoptera stings are the most common trigger of anaphylaxis in PMCD<sup>2, 3</sup>. After re-exposure to Hymenoptera venom these patients also have a higher recurrence rate for developing severe anaphylaxis compared to the general population when they are not treated with venom immunotherapy (VIT) (97.5 vs 50%)<sup>4</sup>. They also have a higher recurrence rate of anaphylaxis after discontinuation VIT<sup>5</sup>. Therefore, in PMCD with established venom allergy, lifelong VIT is recommended<sup>4</sup>. Furthermore, patients with systemic mastocytosis have an increased risk of developing osteopenia/osteoporosis<sup>6</sup>. PMCD encompass systemic mastocytosis (SM) as well as pre-diagnostic forms of SM (pdSM) that do not fulfill the WHO criteria including monoclonal mast cell activation syndrome (MMAS)<sup>7</sup>. The diagnosis of SM is made when 1 major and 1 minor criterium or 3 minor criteria of the World Health Organization (WHO) criteria are fulfilled<sup>8</sup>. Patients with pdSM meet only 1 or 2 minor WHO diagnostic criteria<sup>8-10</sup>. The detection of KIT D816V in extracutaneous cells is a minor criterium<sup>11</sup> and is present in about 90% of patients with systemic mastocytosis (SM)<sup>12</sup>. Because of the increased risk of severe/life-threatening anaphylaxis and comorbidities, early recognition and management of a PMCD is of vital importance. In 2014, The European Competence Network on Mastocytosis (ECNM) has proposed two consensus algorithms for recognition of an underlying PMCD<sup>13</sup>. In adult patients who present with typical maculopapular cutaneous lesions (henceforth called mastocytosis in the skin [MIS])<sup>14</sup>, diagnostic work-up starts with a BM biopsy<sup>13</sup>. In contrast, in patients who experienced anaphylaxis but without MIS, the decision whether to perform a BM biopsy is not always straightforward. In such patients without MIS, the ECNM recommends a BM biopsy when the baseline serum tryptase (bST) is  $> 30$  ng/mL, with a REMA score of  $\geq 2$ <sup>15</sup> or when a KIT D816V mutation in peripheral blood (PB) is detectable<sup>16, 17</sup>. Other scoring tools have been developed to predict clonality in patients with idiopathic anaphylaxis. These are the modified REMA (mREMA) score developed by Gülen<sup>18</sup> et al. and NIH Idiopathic Clonal Anaphylaxis Score (NICAS) developed by Carter et al<sup>19</sup>. Unlike, the REMA and mREMA score, the NICAS incorporates the detection of KIT D816V mutation in PB to augment its' diagnostic

128 accuracy. However, in patients with a suspected PMCD, the indication of BM examination  
129 based on the results of a KIT D816V in PB remains to be established.

130 In this study we investigated whether detection of KIT D816V in PB with a highly sensitive  
131 PCR technique can benefit the decision to perform a BM biopsy in patients with anaphylaxis  
132 without MIS and a bST < 30 ng/mL.

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## Methods

### Patients

Seventy-four patients were recruited from the Immunology/Allergology outpatient clinic of the Antwerp University Hospital between January 2016 and March 2020. All patients experienced severe anaphylaxis as defined by the AAAAI/EAACI guidelines<sup>1, 20</sup>, had no evidence of MIS and a bST < 30 ng/mL. Forty-six patients presented with a REMA-score  $\geq 2$ <sup>15</sup>. Twenty-eight patients had a REMA score < 2 (figure 1): anaphylaxis with severe hypotension together with mucocutaneous symptoms (N = 21); anaphylaxis with a bST  $\geq 20$  ng/mL but < 30 ng/mL (N = 3); or recurrent idiopathic anaphylaxis (N = 4). The diagnosis of SM was made according to the WHO diagnostic criteria<sup>8</sup>. Briefly, these are presence of MC infiltrates of  $\geq 15$  MCs in BM or extracutaneous organs (major), detection of KIT D816V mutation (minor), bST > 20 ng/mL (minor), aberrant expression of CD2/CD25 on MCs (minor) and > 25% spindle shaped MCs (minor)<sup>8, 21</sup>. When patients did not fulfill the WHO criteria, they were assigned to the category of pdSM. This included MMAS when signs of clonality (KIT D816V and/or CD2/CD25) were present and morphologically abnormal MC when spindle shaped MC >25% were found without signs of clonality (from here on described as SSMC).

Modified REMA score<sup>18</sup> and NICAS<sup>19</sup> was calculated retrospectively. Approval for this study was obtained from the local Ethics Committee (2B300201836890).

### Laboratory studies

#### *Baseline serum tryptase*

Baseline serum tryptase was quantified by the FEIA ImmunoCAP (Phadia Thermo Fisher Scientific, Uppsala, Sweden). According to the manufacturer 11.4 ng/mL was considered as the upper limit of normal.

#### *KIT D816V mutation in peripheral blood and bone marrow*

KIT D816V mutation analysis was performed with the PrimePCR™ digital droplet PCR (ddPCR) Mutation Detection Kit Assay containing a primer-probe mix and a ddPCR supermix (Bio-Rad Laboratories Inc, Hercules, California, USA). After preparing reaction mix, droplets were generated in a QX200 droplet generator (Bio-Rad laboratories Inc) and DNA was amplified on a Verity PCR instrument (Life Technologies). Following PCR amplification, the PCR plate was read in a QX200 droplet reader and data were analyzed

using the QuantaSoft analysis software (Bio-Rad Laboratories Inc). This technique has previously been described as a suitable method for KIT D816V testing<sup>22</sup>. In our laboratory, the accuracy and sensitivity of the ddPCR mutation detection was validated on a cohort of systemic mastocytosis patients with cutaneous lesions. According to allele-specific Real-Time PCR, 18 were known weak positive samples (0.01%-2%), 5 known strong positive samples (24-50%) and 13 known negative samples (both bone marrow and peripheral blood). Intra- and inter-assay precision was <10 % coefficient of variation. A strong positive sample was diluted in a negative cell line and was reproducibly detected in a 10<sup>-4</sup> dilution representing 0.008% KIT mutation confirming the intended sensitivity of at least 0.01%. The samples in this study were analyzed at least in duplicate and with a minimal sensitivity of 0.01% positive alleles. In each run all appropriate controls were included.

#### Statistical analysis

All results were expressed as median with range or interquartile range (IQR). Non-parametric Mann–Whitney U test was used to compare bST levels. Paired data of KIT D816V allele burden in BM and PB was compared using Wilcoxon matched-pairs signed rank test. Chi-square test was applied to calculate differences between categorical variables such as sex, triggers of anaphylaxis and clinical features. P-values  $\leq 0.05$  were considered significant. Results were analyzed using PRISM 8 (GraphPad Software, San Diego, USA) and JMP Pro 14 (SAS software, Cary, USA).

## Results

### Patients

After staging, a PMCD was diagnosed in 40 of the 74 subjects (54%). A flowchart of included patients is shown in figure 2. One patient had CD30 positive MCs. Although expression of CD30 positivity is not officially recognized as a minor criterium, its' presence is considered a sign of aberrancy and is, in some cases, the only aberrant marker expressed by clonal mast cells<sup>23-25</sup>. Therefore, this patient was allocated to the pdSM group for further analysis.

Fifty-seven percent of our patients were men and 43% were women with a median age of 60 (range 16-83). No differences in sex and age were found between patients with and without PMCD. Clinical features occurring during anaphylaxis are shown in figure 3. Syncope/presyncope was the most frequent symptom and there was no difference in prevalence of clinical features between PMCD and non PMCD patients except for urticaria that was more prevalent during anaphylaxis of patients without PMCD ( $p = 0.008$ ).

Hymenoptera venom accounted for 70% of the anaphylactic events in patient with PMCD and in 47% of patients without PMCD ( $p = 0.04$ ). Drugs accounted for respectively 18% and 12% of anaphylactic events in patients with and without PMCD ( $p = 0.49$ ). Food was responsible for 2.5% of anaphylactic events in PMCD and 9% of individuals without PMCD ( $p = 0.22$ ). No culprit could be identified in 10% of patients with PMCD and 32% of patients without PMCD ( $p = 0.02$ ) and these events were defined as idiopathic anaphylaxis (figure E1A of the online repository). Multiple anaphylactic events were more frequent in patients without PMCD (53%) than in patients with PMCD (30%,  $p = 0.04$ ) (figure E1B of the online repository).

Full details about study population, triggers of anaphylaxis, confirmatory testing and diagnostic category for each individual patient can be found in table 1.

### Baseline serum tryptase (bST)

Figure 4 shows that median bST was higher for patients with a SM compared to patients with MMAS and SSMC, respectively 13.6 ng/mL, 8.3 ( $p = 0.02$ ) and 8 ng/mL ( $p = 0.009$ ). There was no significant difference between bST from patients with MMAS and SSMC ( $p = 0.79$ ). Patients without a PMCD had a median bST of 4.9 ng/mL, which was lower than values obtained in patients with SM, MMAS or SSMC (respectively  $p < .001$ ,  $p = 0.002$  and 0.04).

Twenty-two of the 74 (30%) patients presented with a bST  $\leq 5$  ng/mL, 35 (47%) patients had a bST  $> 5 - \leq 11.4$  ng/mL, 10 (14%) with a bST between  $> 11.4 - \leq 20.0$  ng/mL and 7 (9%) with a bST between  $> 20 - 30$  ng/mL. The distribution of the different tryptase intervals for patients with and without PMCD are shown in figure E2 of the online repository.

#### Acute serum tryptase

Acute serum tryptase (aST) levels were available in 25 patients. Twenty-two patients met the consensus formula  $[(1.2 \times \text{bST}) + 2]$  for mast cell activation (MCA). Median tryptase increase above the bST was significantly higher in patients with PMCD than in patients without PMCD (91.5 ng/mL vs 8.5 ng/mL,  $p < .001$ ) (figure E3 of the online repository). Three patients without MCA had no evidence of PMCD.

#### KIT D816V mutation in peripheral blood (PB)

KIT D816V in PB was detected in 17 of the 74 patients with a positive allele burden ranging from 0.004-0.34% D816V positive alleles (median = 0.02%). A PMCD was diagnosed in 16 of the 17 patients with KIT D816V in PB. KIT D816V in PB was not detected in 24 patients with a PMCD (figure E4). Thus, a KIT D816V in PB was detectable in only 16 of the 40 (40%) patients with PMCD. Eight of the 16 PMCD patients were classified as SM and the other 8 as pdSM. Further details on the presence of KIT D816V in PB and the different subcategories of PMCD are displayed in figure 5.

#### KIT D816V mutation in peripheral blood (PB) versus bone marrow (BM)

KIT D816V in BM was detected in 22 of the 74 patients with an allele burden ranging from 0.01-0.34% D816V positive alleles (median = 0.04%). Sixteen of the 74 patients had a detectable KIT D816V mutation in both PB and in BM. In 6 patients a KIT D816V mutation was only demonstrable in BM, not in the PB. One patient had a detectable KIT D816V mutation in PB but not in the BM. In this case, the KIT D816V mutation was detected in 0.005% of alleles and was close to the detection limit of the sample. No compact MC infiltrates nor immunophenotypically aberrant mast cells were found and bST was 10.9 ng/mL. Further details on the presence of KIT D816V in BM and the different subcategories of PMCD can be found in figure 5.

Paired data of detectable KIT D816V allele burden in BM and PB was available in 15 patients. Figure 6 shows that the median allele burden in BM (0.05%, range 0.01-0.34) was higher than in PB (0.02%, range 0.005-0.34) ( $p = 0.01$ ). However, there is an overlap between the results of KIT D816V in BM and in PB, especially in patients with higher allele burden.

#### KIT D816V in PB versus bST

In patients with PMCD, there was no significant difference between bST levels in patients with or without KIT D816V mutation in PB (median 11.6 ng/mL [range 4.3-20 ng/mL] vs 8.3 ng/mL [range 4.2-27 ng/mL] [Figure E5 of the online repository]).

#### KIT D816V in PB versus REMA score, mREMA and NICAS

In 32/46 (70%) of patients who presented with a REMA score  $\geq 2$ , the diagnosis of a PMCD was established. Fourteen of these 32 patients (44%) had a detectable KIT D816V in PB. KIT D816V in PB could be detected in 2 of the 8 patients (25%) with a PMCD and a REMA score  $< 2$  (figure 1). Fourteen out of 46 (30%) patients with a REMA score  $\geq 2$  did not display a primary mast cell disorder. Comparison of patients with and without PMCD with a REMA  $\geq 2$ , showed no difference between ratio M/F or specific symptoms (syncope/presyncope, urticaria, angioedema, pruritus). However, median tryptase in the PMCD-/REMA  $\geq 2$  group was 5.9 ng/mL (IQR: 4.3-15.4) and in the PMCD+/REMA  $\geq 2$  group was 9 (IQR: 5.7-20.4) ( $p = 0.01$ ).

In patients with PMCD, mREMA score was positive ( $\geq 2$ ) in 33/40 (70%) individuals. In 14 out of these 33 patients, a KIT D816V mutation in PB was detectable. Five patients with a mREMA  $< 2$  had a PMCD without detectable KIT D816V in PB (figure E6A of the online repository). In patients with PMCD, NICAS was positive ( $\geq 2$ ) in 28/40 (70%) individuals. In 16 out of these 28 patients, a KIT D816V mutation in PB was detectable. Twelve patients with a NICAS  $< 2$  had a PMCD without detectable KIT D816V in PB (figure E6B of the online repository).

Details on frequencies of REMA score, mREMA score, NICAS, and KIT D816V in PB in different sBT intervals are shown in table 2.

## Discussion

Anaphylaxis is a potentially life-threatening condition, especially in patients with an underlying PMCD<sup>26</sup>. Consequently, diagnostic work-up of patients who experienced (severe) anaphylaxis cannot be restricted to the identification of the possible culprit(s). It also necessitates an assessment for an underlying PMCD because missing this condition can have serious consequences. As explained in the introductory paragraph, PMCD patients are at increased risk of recurrence of severe anaphylaxis after re-exposure to Hymenoptera venom when not treated with VIT and also when VIT is stopped after 5 years<sup>4, 5</sup> and of developing osteoporosis<sup>6</sup>. Keeping this in mind, knowing when to perform a BM biopsy for further work-up is essential. In adults who present with MIS and/or a bST  $\geq 30$  ng/mL, this decision is straightforward. These patients should always get BM examination<sup>13</sup>. In contrast, in the cases who do not meet these criteria, this decision might be less obvious. In these patients, detection of KIT D816V in PB could serve as a guidance for performing a BM biopsy<sup>13</sup>. Detection of KIT D816V mutation in PB was developed in 2011. The technique is based on an allele-specific real time quantitative PCR assay that allows quantification of minimally 0.01% KIT D816V mutation-positive cells<sup>17</sup>. Review of the literature shows high detection rates of KIT D816V in PB in patients with PMCD ranging from 85-94%<sup>16, 27</sup>. However, taking a closer look at the data, reveals considerable differences in the test performance between PMCD patients with and without MIS. In the study by Jara-Acevedo et al.<sup>27</sup>, sensitivity of KIT D816V in PB was 92% for patients with MIS but only 66% for patients without MIS. Kristensen et al.<sup>13</sup>, detected KIT D816V in PB of 96% patients with MIS but only in 40% of patients without MIS. In our study, focusing on patients without MIS and a bST  $< 30$  ng/mL, KIT D816V in PB was detected in only 40% of patients with a PMCD. This result reinforces the fact that a negative test outcome is not reliable to exclude a PMCD. Moreover, a closer look at the population who would benefit the most from this assay, that is, patients with a REMA score  $< 2$ <sup>13</sup>, reveals that the result of this assay is of limited value. Actually, in that category only 25% of PMCD patients tested positive. The low detection rate of the KIT D816V in PB in our population, even when using a highly sensitive technique, could have different explanations. The majority of our PMCD patients who experienced anaphylaxis, present with a low MC burden as reflected by a bST  $< 11.4$  ng/mL. Alternatively, the analysis does not cover the entire mutational profile of KIT in PMCD<sup>12, 28</sup>.

However, it is of utmost importance not to overlook the diagnosis of PMCD in this group, since these patients belong to the highest risk group for recurrence of anaphylaxis<sup>4, 29</sup>. Regarding the triggers of anaphylaxis, Hymenoptera venom, in line with previous reports<sup>15</sup> was the most frequent followed by drugs and food. The prevalence of food induced anaphylaxis is similar to other reports<sup>30</sup>, whereas prevalence of immediate drug hypersensitivity was higher than previously published<sup>31</sup>. Interestingly, in our population, patients without a PMCD displayed more multiple anaphylactic events when compared to patients with a PMCD. Furthermore, comparison of aST levels between patients with and without a PMCD showed a significantly higher increase of aST in the first group. Additionally, patients without MCA during the anaphylactic event did not have a PMCD. This result indicates that the suspicion of PMCD should be high in those patients with a pronounced increase of serum tryptase during the acute event.

Nine patients with pdSM only displayed spindle shaped mast cells at diagnostic work-up (figure 2). One could argue that this could be a mechanical artifact of trephine biopsy. However, in these patients more than 25% of the mast cells had a spindle shaped morphology and these findings were confirmed by a trained pathologist. Serum baseline tryptase of these patients was also significantly higher than patients without PMCD but did not differ from sBT levels of MMAS patients. Furthermore, other studies confirm an isolated population of spindle-shaped MC without detection of KIT D816V as a pre-diagnostic form of SM<sup>32</sup>. In a recent study performed by Giannetti et al.<sup>33</sup>, 4 out of 10 patients with a hereditary alpha-tryptasemia (HαT) and mast cell activation symptoms, a variable percentage of spindle shape mast cells without other signs of clonality were found in the bone marrow. In our study 5 of the 9 patients who displayed spindle shaped mast cells >25%, without other signs of clonality had a bST > 8 ng/mL. On the other hand, in 7 out 34 patients without evidence of PMCD a HαT might be suspected because of a bST > 8 ng/mL. A limitation is that those patients were not investigated for presence of multiple copy numbers of TPSAB1 that could be suspected in those patients. To date, the association between HαT, morphological abnormal mast cells and mast cell activation symptoms deserves further investigation. Especially in clinical practice the presence of HαT can be a confounder for suspecting a primary mast cell disorder in patients with elevated sBT.

One patient had a detectable KIT D816V mutation in PB close to the detection limit, but no mutation was detectable in BM. This patient had no other signs of aberrant or clonal MCs. The clinical significance of an isolated detection of KIT D816V in PB is unclear but warrants

further follow up because similar levels of detectable KIT D816V in PB were also found in some of our patients with a diagnosis of ISM.

Scoring systems incorporating different clinical and laboratory parameters have been developed to help predict an underlying PMCD in patients without MIS. The REMA score developed by the Spanish Mastocytosis Network was validated for patients presenting with anaphylaxis due to a wide variety of triggers<sup>15</sup>. In over half of our PMCD patients with a positive REMA score, no KIT D816V in PB was detectable. As stated above, KIT D816V in PB was only detectable in 25% of patients with a PMCD and a negative REMA score. When the mREMA score was applied to our population one additional patient could be identified. On the other hand, our data also reveals a relatively high percentage of REMA score  $\geq 2$  without PMCD. This can be explained by the fact that patients who develop presyncope/syncope without mucocutaneous symptoms during anaphylaxis automatically have a positive REMA score independent of sex or bST. Importantly, bST was significantly lower in patients without PMCD than in patients with a PMCD (5.9 vs 9 ng/mL), indicating that the REMA score performs less in patients with a low bST. In this study, we also examined how NICAS that incorporates detection of KIT D816V in PB, performed in detecting clonal mast cell disorders. NICAS was negative in 30% of our patients. Two possible explanations can be postulated. First, the majority of our patients experienced Hymenoptera venom anaphylaxis, whereas NICAS was validated for idiopathic anaphylaxis. Second, the low frequency of detection of KIT D816V in PB in our population had a negative impact on the NICAS calculation. Admittedly, a limitation of this study is the retrospective calculation of NICAS.

In conclusion, our study shows that in patients who experience anaphylaxis and who present without mastocytosis in the skin and a baseline tryptase below 30 ng/mL, quantification of a KIT D816V mutation in peripheral blood is of limited help in deciding when to perform a BM biopsy. Therefore, KIT D816V in PB mutation analysis should be interpreted together with scoring tools to make a better assessment in identifying patients who should undergo bone marrow biopsy. In patients with low bST (i.e.  $\leq 5$  ng/mL) performing a BM biopsy is debatable and scoring tools alone do not have an optimal performance. A positive KIT D816V result aids the decision to move forward to a BM investigation. On the other hand, in patients with bST between 5 and 30 ng/mL the negative predictive value of KIT D816V is around 40%. This means that we need to optimize tools for identifying the risk of an underlying PMCD, especially in patients with low baseline tryptase.

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## Tables

**Table 1: Demographical data, number of anaphylactic events (#APX), triggers and their diagnostic tests, serum baseline and acute tryptase (bST and aST), detection of KIT D816V in peripheral blood (PB) and bone marrow (BM) and category of mast cell disorder.**

**Table 1A: patients with a primary mast cell disorder after diagnostic work up (PMCD).**

**Table 1B: patients without a primary mast cell disorder after diagnostic work up**

N: patient number; PMCD: primary mast cell disorder; SSMC: population of > 25% spindle shaped mast cells; MMAS: monoclonal mast cell activation syndrome; ISM: indolent systemic; SM with AHN: systemic mastocytosis with an associated hematological neoplasm; mastocytosis; KIT+: KIT D816V positive in bone marrow; CD25+: expression of CD25 on bone marrow mast cells; CD30+: expression of CD30 on bone marrow mast cells; VIT: venom immunotherapy; ST: skin test; BAT: basophil activation test; *recombinant* (r)Ves v: *Vespula vulgaris*; rApi m: *Apis mellifera*; rBet v: *Betula verrucosa*; NA: not available; POS: positive; NEG: negative; M: male, F: female

\* Patient experienced bee-sting induced anaphylaxis 8 years after stopping VIT.

**Table 2. Distribution of REMA score, modified REMA score, NICAS and KIT D816V positive and negative results in different tryptase intervals for patients with and without a primary mast cell disorder (PMCD).**

## Figure legends

**Figure 1. Presence (+) and absence (-) of KIT D816V mutation in peripheral blood (PB) versus the REMA score.**

Panel A represents patients with a REMA score  $\geq 2$ . Panel B represents patients whose clinical presentation of anaphylaxis was less typical for SM and therefore got a REMA score of <2.

**Figure 2. Flowchart of patients the 40 patients with a primary mast cell disorder (PMCD) and their diagnostic subcategories.**

SM: systemic mastocytosis, ISM: indolent systemic mastocytosis, SM-AHN: systemic mastocytosis with an associated hematological neoplasm, pdSM: pre-diagnostic systemic

mastocytosis, MMAS: monoclonal mast cell activation syndrome, SSMC: patients with population of  $\geq 25\%$  spindle shaped mast cells, CD30+ MC: mast cells expressing CD30

\* The MMAS group includes 6 patients with mast cells expressing CD25, 5 patients with KIT D816V mutation in bone marrow, 2 patients with KIT D816V mutation in bone marrow and a baseline serum tryptase of  $\geq 20$  ng/mL, 3 patients with KIT D816V mutation and mast cells expressing CD25, 1 patient with KIT D816V mutation and  $\geq 25\%$  spindle shaped mast cells in the bone marrow biopsy.

**Figure 3. Distribution of different clinical features occurring during anaphylaxis (in patients with and without primary mast cell disorder (PMCD)).**

**Figure 4. Distribution of baseline serum tryptase (bST) levels between patients with systemic mastocytosis (SM), with a pre-diagnostic form of SM (MMAS and SSMC) and without a primary mast cell disorder (PMCD) after bone marrow investigation (no PMCD).**

The red triangle represents the patient with a CD30 positive population of mast cells in his bone marrow biopsy. Because this is a marker of clonality the patient was grouped together with the other patients with monoclonal mast cell activation syndrome (MMAS). Horizontal lines represent median and interquartile range. SSMC: spindle shaped mast cells

**Figure 5. Different categories of primary mast cell disorders (PMCD) and presence or absence of KIT D816V in bone marrow (BM) and/or peripheral blood (PB).**

ISM: indolent systemic mastocytosis; ISM-AHN: indolent systemic mastocytosis with an associated hematological neoplasm; KIT D816V+: patients satisfying only the minor criterium of KIT D816V mutation detected in BM; CD25+: patients with CD25 positive mast cells; SSMC: patients with isolated spindle shaped mast cells; CD30 +: patients with CD30 positive mast cells

**Figure 6. KIT D816V allele burden in bone marrow (BM) and peripheral (PB).**

Median KIT D816V allele burden was 0.02% in PB and 0.05% in BM ( $p = 0.01$ )

**Table 1: Demographical data, number of anaphylactic events (#APX), triggers and their diagnostic tests, serum baseline and acute tryptase (bST and aST), detection of KIT D816V in peripheral blood (PB) and bone marrow (BM) and category of mast cell disorder.**

**Table 1A: patients with a primary mast cell disorder after diagnostic work up (PMCD).**

| N  | Age | Sex | REMA score | # APX | Trigger(s)                                    | Trigger association measurment                                    | bST (ng/mL) | aST (ng/mL) | KIT D816V in PB | KIT D816V in BM | Mast cell disorder category |
|----|-----|-----|------------|-------|-----------------------------------------------|-------------------------------------------------------------------|-------------|-------------|-----------------|-----------------|-----------------------------|
| 1  | 58  | F   | 2          | 1     | Rocuronium                                    | Positive ST to rocuronium, sIgE to morphine and pholcoldine, BAT  | 9.2         | 108         | NEG             | NEG             | SSMC                        |
| 2  | 49  | M   | -2         | 8     | Idiopathic                                    |                                                                   | 5.5         | NA          | NEG             | NEG             | MMAS: CD25+                 |
| 3  | 72  | M   | 4          | 1     | General anesthesia without identified culprit | ST, sIgE and BAT negative for possible culprits                   | 6.4         | 29          | NEG             | NEG             | SSMC                        |
| 4  | 50  | F   | 2          | 1     | Wasp                                          | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5 | 8.4         | 201         | NEG             | POS             | MMAS: KIT+                  |
| 5  | 66  | F   | 2          | 1     | Wasp                                          | Positive sIgE to wasp venom, rVes v 1, rVes v 5                   | 10.7        | 29.6        | POS             | POS             | MMAS: KIT+ and CD25+        |
| 6  | 50  | F   | 2          | 1     | Wasp                                          | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5 | 9.7         | 302         | NEG             | POS             | ISM                         |
| 7  | 62  | M   | 2          | 2     | Idiopathic                                    |                                                                   | 20.5        | NA          | NEG             | NEG             | CD30+ MC                    |
| 8  | 47  | M   | 4          | 4     | Idiopathic                                    |                                                                   | 9           | NA          | POS             | POS             | ISM                         |
| 9  | 68  | M   | 4          | 1     | Wasp                                          | Positive sIgE to wasp venom, rVes v 1, rVes v 5                   | 7.2         | NA          | POS             | POS             | SM with AHN                 |
| 10 | 64  | M   | 4          | 1     | Wasp                                          | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5 | 12.6        | NA          | POS             | POS             | ISM                         |

|    |    |   |    |   |                                                           |                                                                                                                                                                                                                                |      |     |     |     |             |
|----|----|---|----|---|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-----|-----|-----|-------------|
| 11 | 51 | F | 2  | 1 | Bee                                                       | Positive ST to bee venom, sIgE to bee venom                                                                                                                                                                                    | 5.2  | NA  | NEG | NEG | MMAS: CD25+ |
| 12 | 58 | F | -1 | 5 | Apple+ alcohol                                            | Positive sIgE to rBet v 1                                                                                                                                                                                                      | 10.2 | NA  | POS | POS | ISM         |
| 13 | 52 | F | 2  | 1 | General anesthesia without identified culprit             | ST, sIgE and BAT negative for possible culprits                                                                                                                                                                                | 8.8  | 429 | NEG | NEG | SSMC        |
| 14 | 61 | M | 2  | 1 | General anesthesia                                        | ST, sIgE and BAT negative for possible culprits . Because of the temporal relationship with infusion, high molecular weight heparin was considered as possible culprit based on its ability of inducing complement activation. | 20   | 101 | POS | POS | MMAS: KIT+  |
| 15 | 79 | M | 4  | 4 | Gelofusine, wasp                                          | Positive ST to gelofusin, sIgE gelatin, BAT gelofusin. Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                                                                                                       | 10.5 | 102 | NEG | NEG | SSMC        |
| 16 | 45 | M | 5  | 2 | Wasp                                                      | Positive ST to wasp venom, IgE to wasp venom, rVes v 1, rVes v 5                                                                                                                                                               | 15.3 | NA  | POS | POS | ISM         |
| 17 | 61 | F | 0  | 1 | Temporal association with Levobupivacaine and Ropivacaine | ST, sIgE and BAT negative for possible culprits. Tolerance for Lidocaine.                                                                                                                                                      | 21.2 | NA  | NEG | NEG | SSMC        |
| 18 | 34 | M | 4  | 1 | Wasp                                                      | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                                                                                                                                                              | 5.7  | NA  | NEG | NEG | MMAS: CD25+ |
| 19 | 47 | F | -1 | 4 | Bee, VIT bee*                                             | Positive ST to bee venom, sIgE to bee venom and rApi m 1                                                                                                                                                                       | 4.5  | NA  | NEG | NEG | SSMC        |

|    |    |   |    |   |                                |                                                                                                                                                                                                                                                                            |      |     |     |     |                      |
|----|----|---|----|---|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-----|-----|-----|----------------------|
| 20 | 61 | F | 2  | 1 | Unknown insect                 | Negative ST, sIgE to bee venom, wasp venom, rVes v 1, rVes v 5, rApi m 1 and BAT                                                                                                                                                                                           | 10.5 | NA  | POS | POS | MMAS: KIT+ and CD25+ |
| 21 | 33 | M | 4  | 2 | Wasp                           | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                                                                                                                                                                                                          | 8.3  | NA  | POS | POS | MMAS: KIT+           |
| 22 | 63 | F | 3  | 1 | Wasp                           | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                                                                                                                                                                                                          | 16.7 | 187 | POS | POS | ISM                  |
| 23 | 54 | M | 4  | 1 | Wasp                           | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                                                                                                                                                                                                          | 8    | NA  | NEG | NEG | SSMC                 |
| 24 | 44 | F | 2  | 2 | Wasp                           | Positive ST to wasp venom, sIgE to wasp venom, rVes v 5                                                                                                                                                                                                                    | 5.8  | NA  | NEG | NEG | MMAS: KIT+ and SSMC  |
| 25 | 45 | M | 2  | 1 | Wasp                           | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                                                                                                                                                                                                          | 27   | NA  | NEG | POS | ISM                  |
| 26 | 53 | M | 1  | 2 | Idiopathic                     |                                                                                                                                                                                                                                                                            | 21   | 50  | NEG | NEG | MMAS: CD25+          |
| 27 | 72 | M | 1  | 3 | Amoxicillin/ Moxifloxacin (2x) | Clear history with onset of events within 1 hour after exposure. ST and sIgE negative for penicillins. possibly fals negative due to long time between indexreaction and testing. No reliable skin testing for moxifloxacin available. Drug challenges were not performed. | 4.2  | NA  | NEG | NEG | SSMC                 |
| 28 | 47 | M | 7  | 1 | Wasp                           | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                                                                                                                                                                                                          | 25.5 | NA  | NEG | POS | ISM                  |
| 29 | 49 | F | 2  | 1 | Wasp                           | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                                                                                                                                                                                                          | 8.2  | NA  | NEG | NEG | MMAS: CD25+          |
| 30 | 70 | M | 4  | 1 | Wasp                           | Positive ST to wasp venom, sIgE to rVes v 1, rVes v 5                                                                                                                                                                                                                      | 5.8  | NA  | NEG | NEG | MMAS: CD25+          |
| 31 | 52 | F | -1 | 1 | Wasp                           | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                                                                                                                                                                                                          | 11   | NA  | NEG | POS | ISM                  |

|    |    |   |    |   |      |                                                                   |      |      |     |     |                      |
|----|----|---|----|---|------|-------------------------------------------------------------------|------|------|-----|-----|----------------------|
| 32 | 66 | F | -1 | 1 | Wasp | Positive ST to wasp venom, sIgE to wasp venom, rVes v 5           | 13.6 | NA   | POS | POS | ISM                  |
| 33 | 37 | M | 4  | 1 | Bee  | Positive ST to bee venom, sIgE to bee venom and rApi m 1          | 7.8  | 21.4 | POS | POS | MMAS: KIT+ and CD25+ |
| 34 | 64 | M | 4  | 1 | Wasp | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5 | 4.3  | NA   | POS | POS | MMAS: KIT+           |
| 35 | 67 | F | 2  | 1 | Wasp | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5 | 13.9 | NA   | POS | POS | MMAS: KIT+           |
| 36 | 55 | M | 4  | 1 | Wasp | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5 | 8    | NA   | NEG | NEG | SSMC                 |
| 37 | 56 | M | 4  | 1 | Wasp | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5 | 5.8  | NA   | NEG | NEG | MMAS: CD25+          |
| 38 | 64 | F | 2  | 1 | Wasp | Positive ST to wasp venom, sIgE to wasp venom, rVes v 5           | 12.5 | NA   | POS | POS | MMAS: KIT+           |
| 39 | 64 | F | 3  | 1 | Wasp | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5 | 15.4 | NA   | NEG | POS | ISM                  |
| 40 | 60 | M | 5  | 2 | Wasp | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5 | 17.2 | NA   | POS | POS | ISM                  |

**Table 1B: patients without a primary mast cell disorder after diagnostic work up**

| <b>N</b> | <b>Age</b> | <b>Sex</b> | <b>REMA score</b> | <b># APX</b> | <b>Trigger(s)</b> | <b>Trigger association measurment</b>                                                       | <b>bST (ng/mL)</b> | <b>aST (ng/mL)</b> | <b>KIT D816V in PB</b> | <b>KIT D816V in BM</b> | <b>Mast cell disorder category</b> |
|----------|------------|------------|-------------------|--------------|-------------------|---------------------------------------------------------------------------------------------|--------------------|--------------------|------------------------|------------------------|------------------------------------|
| 1        | 60         | M          | 1                 | 4            | Idiopathic        |                                                                                             | 6.7                | NA                 | NEG                    | NEG                    | no PMCD                            |
| 2        | 16         | F          | 2                 | 1            | Idiopathic        |                                                                                             | 3.1                | NA                 | NEG                    | NEG                    | no PMCD                            |
| 3        | 70         | F          | -1                | 3            | Idiopathic        |                                                                                             | 2.9                | 6.6                | NEG                    | NEG                    | no PMCD                            |
| 4        | 63         | M          | 1                 | 1            | Wasp              | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                           | 9.1                | NA                 | NEG                    | NEG                    | no PMCD                            |
| 5        | 58         | F          | -4                | 6            | Idiopathic        |                                                                                             | 3.4                | 3.9                | NEG                    | NEG                    | no PMCD                            |
| 6        | 60         | M          | 4                 | 1            | Wasp              | Positive ST to wasp venom, sIgE to wasp venom, rVes v1, rVes v 5                            | 2.2                | NA                 | NEG                    | NEG                    | no PMCD                            |
| 7        | 54         | M          | 4                 | 1            | Wasp              | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                           | 5.9                | 16.3               | NEG                    | NEG                    | no PMCD                            |
| 8        | 38         | F          | 2                 | 2            | Wasp, VIT wasp    | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                           | 4.5                | 5.3                | NEG                    | NEG                    | no PMCD                            |
| 9        | 56         | F          | -3                | 2            | Nitrofurantoin    | Clear history with onset of symptoms within 1 hour after exposure. No ST or sIgE available. | 20.3               | 30.3               | NEG                    | NEG                    | no PMCD                            |
| 10       | 78         | M          | -2                | 2            | Idiopathic        |                                                                                             | 10.3               | 90                 | NEG                    | NEG                    | no PMCD                            |

|    |    |   |    |   |                                               |                                                                                             |      |      |     |     |         |
|----|----|---|----|---|-----------------------------------------------|---------------------------------------------------------------------------------------------|------|------|-----|-----|---------|
| 11 | 33 | F | -1 | 1 | Oxytocin                                      | Clear history with onset of symptoms within 1 hour after exposure. Positive ST to oxytocin  | 5.8  | NA   | NEG | NEG | no PMCD |
| 12 | 30 | F | -1 | 3 | Idiopathic                                    |                                                                                             | 4.3  | NA   | NEG | NEG | no PMCD |
| 13 | 68 | M | 1  | 1 | Wasp                                          | Positive ST to wasp venom, sIgE to wasp venom                                               | 3.6  | 10   | NEG | NEG | no PMCD |
| 14 | 71 | M | 4  | 1 | General anesthesia without identified culprit | ST, sIgE and BAT negative for possible culprits                                             | 10.9 | NA   | POS | NEG | no PMCD |
| 15 | 70 | M | 1  | 2 | Ibuprofen                                     | Clear history with onset of symptoms within 1 hour after exposure. No ST or sIgE available. | 3.6  | NA   | NEG | NEG | no PMCD |
| 16 | 67 | M | 1  | 1 | Wasp                                          | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                           | 6.8  | 40.4 | NEG | NEG | no PMCD |
| 17 | 57 | F | -1 | 3 | Idiopathic                                    |                                                                                             | 3    | NA   | NEG | NEG | no PMCD |
| 18 | 63 | M | 4  | 1 | Wasp                                          | Positive sIgE to wasp venom, rVes v 1, rVes v5                                              | 7    | NA   | NEG | NEG | no PMCD |
| 19 | 62 | M | 1  | 1 | Wasp                                          | Positive ST to wasp venom, sIgE to wasp venom, rVes v 5                                     | 3.4  | NA   | NEG | NEG | no PMCD |
| 20 | 72 | M | 1  | 2 | Wasp                                          | Positive ST to wasp venom, sIgE to wasp venom, rVes v 1, rVes v 5                           | 4.8  | 54.9 | NEG | NEG | no PMCD |
| 21 | 80 | M | 2  | 4 | Wheat +exercise                               | Positive sIgE to Omega-5-gliadine and wheat                                                 | 15.5 | NA   | NEG | NEG | no PMCD |
| 22 | 55 | M | 4  | 1 | Wasp                                          | Positive sIgE to wasp venom,                                                                | 4.8  | 13.1 | NEG | NEG | no      |

|    |    |   |    |   |                |                                                                                                            |      |      |     |     |         |
|----|----|---|----|---|----------------|------------------------------------------------------------------------------------------------------------|------|------|-----|-----|---------|
|    |    |   |    |   |                | rVes v 1, rVes v 5                                                                                         |      |      |     |     | PMCD    |
| 23 | 60 | F | 2  | 1 | Unknown insect | Clear history with onset of symptoms within 1 hour after exposure to insect. Event more than 40 years ago. | 6.8  | NA   | NEG | NEG | no PMCD |
| 24 | 83 | M | 1  | 2 | Idiopathic     |                                                                                                            | 3.1  | 6.7  | NEG | NEG | no PMCD |
| 25 | 59 | M | 1  | 2 | Wheat, Treenut | Positive ST to hazelnut, sIgE to peanut, hazelnut, almond, wheat and Omega-5-gliadin                       | 5.2  | NA   | NEG | NEG | no PMCD |
| 26 | 67 | M | 4  | 4 | Idiopathic     |                                                                                                            | 3.9  | 10.5 | NEG | NEG | no PMCD |
| 27 | 23 | F | -1 | 4 | Idiopathic     |                                                                                                            | 3.7  | NA   | NEG | NEG | no PMCD |
| 28 | 58 | F | 2  | 1 | Wasp           | Positive sIgE to wasp venom, rVes v 1, rVes v 5                                                            | 9.9  | NA   | NEG | NEG | no PMCD |
| 29 | 61 | F | 2  | 3 | Fig            | Positive sIgE to fig                                                                                       | 6    | NA   | NEG | NEG | no PMCD |
| 30 | 71 | M | 1  | 1 | Wasp           | Positive sIgE to wasp venom, rVes v 1, rVes v 5                                                            | 4.3  | NA   | NEG | NEG | no PMCD |
| 31 | 64 | F | 2  | 2 | Wasp, bee      | Positive sIgE to wasp venom, rVes v 1, rVes v 5, bee venom, Api m 1                                        | 4.9  | NA   | NEG | NEG | no PMCD |
| 32 | 73 | M | 2  | 1 | Wasp           | Positive sIgE to wasp venom, rVes v 1, rVes v 5                                                            | 15.2 | 15.9 | NEG | NEG | no PMCD |
| 33 | 38 | F | -1 | 1 | Wasp           | Positive sIgE to wasp venom, rVes v 1, rVes v 5                                                            | 4.9  | NA   | NEG | NEG | no PMCD |
| 34 | 74 | M | -2 | 3 | Idiopathic     |                                                                                                            | 4.7  | 13.2 | NEG | NEG | no PMCD |

N: patient number; PMCD: primary mast cell disorder; SSMC: population of > 25% spindle shaped mast cells; MMAS: monoclonal mast cell activation syndrome; ISM: indolent systemic; SM with AHN: systemic mastocytosis with an associated hematological neoplasm; mastocytosis; KIT+: KIT D816V positive in bone marrow; CD25+: expression of CD25 on bone marrow mast cells; CD30+: expression of CD30 on bone marrow mast cells; VIT: venom immunotherapy; ST: skin test; BAT: basophil activation test; *recombinant* (r)Ves v: *Vespula vulgaris*; rApi m: *Apis mellifera*; rBet v: *Betula verrucosa*; NA: not available; POS: positive; NEG: negative; M: male, F: female

\* Patient experienced bee-sting induced anaphylaxis 8 years after stopping VIT.

**Table 2. Distribution of REMA score, modified REMA score, NICAS and KIT D816V positive and negative results in different tryptase intervals for patients with and without a primary mast cell disorder (PMCD).**

| <b>PMCD<br/>(N=40)</b>    | <b>REMA score</b> |           | <b>Modified REMA<br/>score</b> |           | <b>NICAS</b> |           | <b>KIT D816V in PB</b> |          |
|---------------------------|-------------------|-----------|--------------------------------|-----------|--------------|-----------|------------------------|----------|
| <b>sBT<br/>(ng/mL)</b>    | <b>&lt;2</b>      | <b>≥2</b> | <b>&lt;2</b>                   | <b>≥2</b> | <b>&lt;2</b> | <b>≥2</b> | <b>-</b>               | <b>+</b> |
| <b>≤5</b>                 | 2                 | 1         | 2                              | 1         | 1            | 2         | 2                      | 1        |
| <b>&gt;5-≤11.4</b>        | 3                 | 20        | 3                              | 20        | 10           | 13        | 16                     | 7        |
| <b>&gt;11.4-≤20</b>       | 1                 | 7         | 1                              | 7         | 0            | 8         | 1                      | 7        |
| <b>&gt;20-30</b>          | 2                 | 4         | 1                              | 5         | 1            | 5         | 5                      | 1        |
| <b>Total</b>              | 8                 | 32        | 7                              | 33        | 12           | 28        | 24                     | 16       |
| <b>no PMCD<br/>(N=34)</b> | <b>REMA score</b> |           | <b>Modified REMA<br/>score</b> |           | <b>NICAS</b> |           | <b>KIT D816V in PB</b> |          |
| <b>sBT<br/>(ng/mL)</b>    | <b>&lt;2</b>      | <b>≥2</b> | <b>&lt;2</b>                   | <b>≥2</b> | <b>&lt;2</b> | <b>≥2</b> | <b>-</b>               | <b>+</b> |
| <b>≤5</b>                 | 13                | 6         | 13                             | 6         | 10           | 9         | 19                     | 0        |
| <b>&gt;5-≤11.4</b>        | 6                 | 6         | 6                              | 6         | 8            | 4         | 11                     | 1        |
| <b>&gt;11.4-≤20</b>       | 0                 | 2         | 0                              | 2         | 0            | 2         | 2                      | 0        |
| <b>&gt;20-30</b>          | 1                 | 0         | 1                              | 0         | 1            | 0         | 1                      | 0        |
| <b>Total</b>              | 20                | 14        | 20                             | 14        | 19           | 15        | 33                     | 1        |

