

## Short communication

# How much specific is the association between hymenoptera venom allergy and mastocytosis?

**Background:** The preferential association of mastocytosis with hymenoptera sting reactions is well known, but there is no data on the prevalence of clonal mast cell disorders in subjects with severe systemic reactions due to foods or drugs.

**Methods:** Patients with food- or drug-induced severe systemic reactions, including anaphylaxis, and increased serum tryptase were studied for the presence of mastocytosis, and compared with a population of patients with hymenoptera allergy. The aetiological role of foods or drugs was assessed according to current recommendations. Systemic reactions were graded in severity according to the procedure described by Mueller. Serum tryptase was considered increased if the level was  $> 11.4$  ng/ml. Subjects with increased tryptase had dermatological evaluation and Bone marrow (BM) aspirate-biopsy, which included histology/cytology, flow cytometry and detection of *KIT* mutations.

**Results:** A total of 137 subjects (57 male, mean age 42 years) were studied. Of them, 86 proved positive for drugs and 51 for foods. Overall, out of 137 patients, only nine (6.6%) had a basal tryptase  $> 11.4$  ng/ml, and only two (1.5%) were diagnosed with mastocytosis. This was clearly different from patients with hymenoptera allergy, where 13.9% had elevated tryptase and 11.1% had a clonal mast cell disorder.

**Conclusion:** The association of clonal mast cell disorders with hymenoptera allergy seems to be more specific than that with food- or drug-induced systemic reactions.

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Mastocytosis (MC) is a heterogeneous disorder characterized by the proliferation and accumulation of mast cells in several organs and tissues. Mastocytosis can either be limited to the skin or involve extracutaneous organs (systemic MC), mostly (BM) (1). The diagnosis of systemic MC requires the presence of multifocal dense MC infiltrates in BM or other extracutaneous organs plus one minor criterion, or, alternatively, three minor criteria. The minor criteria are (i) abnormal morphology of extracutaneous MC infiltrates (spindle-shaped), (ii) increased serum tryptase level ( $> 20$  ng/ml), (iii) expression of CD2 and/or CD25 on BM mast cells and (iv) *KIT* mutation at codon 816 in extracutaneous organs (2, 3). When only one (excluding raised tryptase level) or two of the minor criteria for systemic MC are met, the disease is then termed monoclonal mast cell activation syndrome (MMAS). Serum tryptase, a mast cell specific product, is

persistently elevated in MC and it is, therefore, included in the diagnostic criteria (1).

In MC several stimuli, including the IgE-mediated reaction, can massively activate mast cells and provoke severe systemic symptoms, including anaphylaxis. This is well known for hymenoptera venom allergy (HVA), where MC has been reported to occur with a relatively high frequency (4, 5). It has been recently shown that 88% of patients with anaphylaxis due to HVA and raised tryptase had indeed a clonal mast cell disorder (MC or MMAS) (6). Similarly to what happens with HVA, it can be hypothesized that also in the case of systemic reactions due to other causes (e.g. foods or drugs), a mast cell clonal disorder occurs with a certain frequency. On the other hand, there is so far no data concerning the presence of MC in food- or drug-induced systemic reactions. For this reason, we assessed the presence of

clonal mast cell disorders in patients with increased serum tryptase and severe systemic reactions provoked by foods or drugs, and compared them with an already studied population of patients with HVA (6).

## Methods

Consecutive patients referred for severe systemic reactions, including anaphylaxis, possibly due to foods or drugs, underwent the standard diagnostic procedures to confirm the causal role of the suspected allergen, and had serum tryptase measured. Subjects with a raised tryptase, after informed consent, had a BM examination to detect the presence of clonal mast cell disorders. The control patients were those with severe systemic reactions following hymenoptera stings and with increased tryptase.

Based on the clinical history, the severity of systemic reaction was graded according to the procedure described by Mueller (7), to have a severity score comparable with that of HVA. Grade IV included full-blown anaphylaxis, defined as a serious multisystemic allergic reaction of rapid onset (8) and requiring adrenaline. The measurement of serum tryptase was performed at least 2 weeks after the last episode, using an immunofluorimetric assay (UniCap 250 Phadia, Uppsala, Sweden), with the threshold value set at 11.4 ng/ml (6, 9). Raised tryptase levels had to be confirmed in another blood sample 2 months later. For food allergy, the diagnostic procedure involved skin prick test with commercial extracts and prick-by-prick with fresh foods. For drug allergy, the following tests were performed: skin prick test and intradermal test for betalactams and/or provocation test for anti-inflammatory agents, according to recommendations of the European Network for Drug Allergy (ENDA) (10, 11), or skin prick test for Iodinated Contrast Media and anaesthetics (12). Subjects with increased tryptase level, after informed consent, underwent dermatological evaluation and BM aspiration and biopsy. Immunohistological and morphological analyses were carried out on BM biopsies and smears to detect mast cell abnormalities according to guidelines (13). A 5-antibody immunofluorimetric evaluation of BM was also performed to identify the expression of CD25 and CD2 on CD117+/CD45+/CD34 cells (BD Biosciences, Milan, Italy). Finally, detection of c-KIT mutations in BM samples was carried out by PCR assays (14). The data of the above patients were compared with those of 244 subjects with hymenoptera venom grade III and IV reactions, included in a previous study. Statistical comparisons for clinical characteristics were made by the Fischer exact test, whereas tryptase levels were analysed by Student's *t*-test.

## Results

A total of 137 patients (57 male, mean age 42 years, age range 5–79) with severe systemic reactions (grade III–IV) due to foods and/or drugs were studied between January 2007 and December 2008. There were 40 grade III and 97 grade IV reactions, the latter including 52 full-blown anaphylaxes treated with adrenaline. Out of the 137 subjects, 86 proved positive for the diagnostic tests for drugs (betalactams, quinolones, anaesthetics, contrast media or anti-inflammatory agents), and 51 for foods (shellfish, nuts, eggs, vegetables). Four of the patients with drug hypersensitivity also proved positive for foods. Out of 137 patients, nine (6.6%) had a basal tryptase > 11.4 ng/ml, seven of whom underwent BM evaluation. The BM biopsy was not performed in one boy (8 years, ceftriaxone allergy) for ethical concerns, and in one woman (63 years, chlorexidine allergy) who refused the procedure. Only two (1.5%) subjects were finally diagnosed to have systemic MC, one with skin involvement histologically confirmed. No case of MMAS was documented. In two patients, only an increase of not-clustered mast cells, with normal morphology and phenotype, was observed.

The characteristics of subjects with increased tryptase level are reported in Table 1. The observed rate of patients with increased tryptase and MC clearly differed from that of the HVA population used for comparison, where 13.9% had elevated tryptase and 11.1% had MC/MMAS (6). Of note, subjects with adverse reactions to drugs had overall more severe reactions than patients with HVA or food hypersensitivity. The comparative data of the populations are detailed in Table 2.

## Discussion

The association between HVA and MC has been already identified, as well as the fact that MC is a risk factor for more severe reactions in HVA patients (15, 16). Serum tryptase has been suggested to be a valuable marker of

Table 1. Characteristics of the patients with systemic reaction from drugs/foods and increased serum tryptase

| Pt no. | Age | Sex | Grade of reaction | Causal agent | Serum tryptase ng/mL | BM biopsy (major criteria) | BM mast cells CD25 + and/or CD2+ | Activating KIT mutation (BM) | Spindle-shaped mast cells (>25%) | MC disorder                |
|--------|-----|-----|-------------------|--------------|----------------------|----------------------------|----------------------------------|------------------------------|----------------------------------|----------------------------|
| 1      | 25  | M   | IV                | Food         | 11.7                 | Pos                        | Pos                              | Pos                          | Pos                              | Systemic MC                |
| 2      | 38  | M   | III               | Food         | 14.9                 | Neg                        | Neg                              | Neg                          | Neg                              | —                          |
| 3      | 37  | F   | IV                | Drug +Food   | 13.2                 | Pos                        | Pos                              | Pos                          | Pos                              | Systemic MC with skin inv. |
| 4      | 47  | F   | III               | Drug         | 16.1                 | Neg                        | Neg                              | Neg                          | Neg                              | —                          |
| 5      | 38  | M   | IV                | Drug         | 19.1                 | Neg                        | Neg                              | Neg                          | Neg                              | —                          |
| 6      | 71  | F   | IV                | Drug         | 19.5                 | Neg                        | Neg                              | Neg                          | Neg                              | —*                         |
| 7      | 65  | M   | IV                | Drug         | 20.1                 | Neg                        | Neg                              | Neg                          | Neg                              | —*                         |
| 8      | 63  | F   | IV                | Drug         | 26.9                 | n.d.                       | n.d.                             | n.d.                         | n.d.                             | —                          |
| 9      | 8   | M   | IV                | Drug         | 26.1                 | n.d.                       | n.d.                             | n.d.                         | n.d.                             | —                          |

n.d., Not done, BM, Bonemarrow, MC, Mastocytosis.

\*Only increased normal mast cells.

Table 2. Characteristics of the patients with food or drug hypersensitivity and with HVA

|                                                     | HVA            | Food allergy  | Drug allergy  |
|-----------------------------------------------------|----------------|---------------|---------------|
| <i>N</i>                                            | 244            | 51            | 86†           |
| Male/female                                         | 173/71         | 29/22*        | 28/58**       |
| Mean age                                            | 43             | 43            | 42            |
| Grade III (%)                                       | 120 (49.2)     | 27 (52.9%)    | 13 (15.1%)*   |
| Grade IV (%)                                        | 124 (50.8)     | 24 (47.1%)    | 73 (84.9%)*   |
| Full-blown anaphylaxis (%)                          | 59 (24.2)      | 17 (33.3%)    | 35 (40.7%)*   |
| Tryptase ng/mL, mean $\pm$ SD                       | 7.7 $\pm$ 10.8 | 4.9 $\pm$ 2.6 | 6.6 $\pm$ 4.8 |
| Raised serum tryptase (%)                           | 34 (13.9)      | 2 (3.9%)*     | 7 (8.1)       |
| Clonal mast cell disorder in BM tested patients (%) | 27/28 (96.4)   | 1/2 (50)*     | 1/5 (20.0)**  |
| Clonal mast cell disorder (%)                       | 27/244 (11.1)  | 1/51 (2.0)*   | 1/86 (1.2)**  |

HVA, hymenoptera venom allergy.

†Four of these patients also proved positive for food.

The asterisks indicate significant differences (\* $P < 0.05$ , \*\* $P < 0.01$ ) vs HVA patients.

the presence of MC is these patients. Of note, the IgE-mediated mechanism is preferential, but not mandatory, to elicit systemic reactions after hymenoptera stings (17). Based on this background, it could be expected that other situations, such as food or drug hypersensitivity, with systemic reactions and/or anaphylaxis, could also hide an underlying mast cell clonal disorder. To verify this hypothesis, we performed an extensive BM study in patients with severe systemic reactions to food/drugs and raised tryptase level. Our data showed that the prevalence of MC in food- or drug-hypersensitive patients is far lower than that in patients with HVA, although the severity of reactions was comparable, and even greater for drug hypersensitivity. Of note, the cut-off value for tryptase at 11.4 ng/ml allowed identifying the two subjects with MC, which would have been missed by using the standard threshold of 20 ng/ml. It is true that in our case series, the vast majority of patients with normal tryptase were not studied for the presence of MC, and

therefore additional important data could be have missed. Certainly, it would be relevant to know the prevalence of MC in all subjects with anaphylaxis, but the diagnosis would require an invasive procedure (BM biopsy), with no real indication, and consequent ethical concerns. Nonetheless, with this limitation, the association of mastocytosis with severe systemic reactions and elevated basal tryptase seems to be, for drugs- or food-induced systemic reactions, not as strong as for HVA, at least from an epidemiological viewpoint. This would, in turn, suggest some specificity in the association between MC and HVA, deserving more detailed mechanistic investigation.

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