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Two cases of elevated tryptase in abdominal aortic aneurysm

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Key words

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Summary

Introduction: *From the literature, patients with a history of anaphylaxis to hymenoptera venom and positive specific IgE have shown a correlation between elevated tryptase levels and two clinical situations: systemic mastocytosis and an increased risk of reactions to venom immunotherapy or hymenoptera sting. Other clinical scenarios could explain elevated tryptase levels.* ***Material and Methods:*** *A 67 year old male (P1) and a 77 year old male (P2) were evaluated for previous severe anaphylaxis to hymenoptera sting. They underwent standard diagnostic work-up for hymenoptera venom allergy. Having found elevated tryptase levels, these were followed by a bone marrow biopsy to rule out systemic mastocytosis.* ***Results:*** *P1: specific IgE and skin tests were positive for Vespula species; tryptase 52.8 ng/ml; P2: specific IgE and skin tests were positive for Vespa cabro and tryptase 153 ng/ml. Bone marrow biopsy results were negative for mastocytosis. We carried out magnetic resonance imaging, in P1 to better characterize the severe osteoporosis and in P2 because during physical examination a pulsating mass had been identified in the mesogastrium, and an aneurysm of the abdominal aorta which required surgical intervention in both patients was detected. Eight months after surgery, tryptase levels had diminished significantly (P1: 11.6 ng/ml and P2: 14.5 ng/ml).* ***Discussion:*** *The elevated tryptase levels were correlated to abdominal aneurysm in both patients. In fact, post-surgery tryptase levels dramatically decreased. These two cases demonstrate that high tryptase levels in subjects with a history of hymenoptera venom anaphylaxis can be associated to undiagnosed aneurysmatic disease.*

Abbreviations

HV: hymenoptera venom
SM: systemic mastocytosis
BM: bone marrow
AAA: abdominal aortic aneurysms
VIT: venom immunotherapy

Tryptase is a neutral serine protease secreted by mast cells during anaphylaxis, and invariably elevated in mastocytosis. Recently, an increase in baseline tryptase levels has been indicated in hymenoptera venom (HV) allergy in association with different clinical features. First of all, hymenoptera anaphylaxis has been frequently described in patients with cutaneous mastocytosis (1,2), and more recently in patients with indolent systemic mastocytosis

(SM) without cutaneous involvement (3). This association is comprehensible, given that the hymenoptera stings can cause anaphylaxis in mastocytosis patients even if not allergic. Secondly, an increase in baseline tryptase level (5.2 ng/mL) has been associated with a higher risk of severe anaphylaxis to hymenoptera stings or during venom immunotherapy (VIT) (4). Up to now, however, there has been no clear scientific explanation to justify the aforementioned relationship between venom allergy and tryptase increase in patients without mastocytosis. In some patients, however, a clonal mast cell disorder (5) has been recently described, putting, once more, the mast cell pathology at the basis of the tryptase behavior in HV allergic patients. Moreover, it has been at least a decade since mast cells have been recognized to be involved in cardiovascular diseases (6), and since then elevated tryptase titers have been associated with acute coronary syndrome (7), in some cases in association with allergic reactions in the so called Kounis syndrome (8). This is not surprising given that tryptase is directly involved in atherosclerotic plaque development, given its ability to activate protease zymogens (9). Moreover, different authors have recently demonstrated that the number of mast cells was increased in human AAA even more significantly than in atherosclerotic lesions, and that their number correlated with the diameters of the AAA (10), thus confirming a direct participation of mast cells in AAA formation, as recently demonstrated in several experimental studies on animal models (11). Furthermore, in a cohort study a significantly higher serum baseline tryptase level was found in AAA patients (12,13). All of these observations led us to infer that the meaning of the increased basal levels of tryptase in HV allergy could be due to associated pathologies that have yet to be fully elucidated. Herein we report the cases of two patients with HV anaphylaxis and very high serum baseline level of tryptase, in whom a suspicion of SM had been advanced. A complete diagnostic work up was applied in order to confirm this diagnosis. In both patients SM was excluded and an abdominal aortic aneurysm (AAA) was diagnosed. The patients were two males, one 67 year old (P1) and the other 77 year old (P2). Both patients were admitted to our Centre for a previous severe anaphylactic reactions to yellow jacket stings, that had occurred about one year before with cardiovascular, cutaneous and respiratory involvement (Muller grade III); both had been treated with epinephrine injection with complete regression of symptoms after a few hours. Both patients were not affected by chronic renal failure inasmuch as renal function was normal. Routine diagnostic work-up was performed for HV allergy. Skin prick tests and intradermal tests were positive for Vespula species (P1) and Vespa crabro (P2). About one year after the anaphylactic reaction the measurement of basal serum tryptase was 52.8 ng/mL (P1) and 153 ng/mL (P2), respectively (normal tryptase value < 5 ng/mL). Thus, bone marrow (BM) biopsy was performed and histologycal, immunohistochemical, phenotypic and morphologic BM examinations were carried out to ascertain the presence of SM. KIT and PDGFRα gene mutations were investigated in all of the BM samples. Sanger sequencing was used to detect the presence of potential mutations in exons 9, 11, 13 and 17 of the Kit gene and in exons 12, 14 and 18 of the PDGFRα gene. Moreover, p.D816V mutation research was performed using a mutant-enriched polymerase chain reaction that blocks the wild-type component and amplifies only the mutated component. Such an approach allows to reveal the presence of very low quantities of mutated DNA with an extremely high sensitivity (0.01% of mutant versus wild-type allele). International diagnostic criteria for the diagnosis of SM were employed, in particular we searched for the presence of multi-focal, dense mast cell infiltration (> 15 mast cells in aggregates) in BM samples and/or in extracutaneous organs (major criteria). As minor criteria we looked for: the presence of D816V KIT mutation in BM, blood, or extracutaneous tissues; the baseline serum tryptase concentration > 20 ng/mL; the expression of KIT plus CD2 and/or CD25 in mast cells from BM, and the presence > 25% of mast cells with atypical or spindle shape. The diagnosis of SM has to be made when either 1 major plus 1 minor or 3 minor criteria are present (14). Mast cell phenotypes were detected and analyzed by flow cytometry, using FACS Canto II flow cytometer (Becton Dickinson, san Jose, CA, USA). Acquisition and analysis were performed by means of Diva Software (Becton Dickinson). We excluded the diagnosis of SM for both patients on the basis of the negativity of the aforementioned criteria. Of note, BM biopsy of P2 revealed a 17 exon mutation of the c-Kit gene with a silent nucleotide substitution (c.2394 C > T; P.lle798lle) that, being not clinically significant, was considered negative and, using flow cytometry, a 0.15% of mast cells CD2/CD25 positive that, given the low percentage, was considered not significant. Subsequently, magnetic resonance imaging was performed in P1 to better characterize the severe osteoporosis detected by bone mineral density test, and in P2 because during physical examination a pulsating mass had been identified in the mesogastrium and the patient complained recurrent episodes of dyspnea. AAAs were detected in both patients with a diameter of 58 mm (P1) and of 54 mm (P2). Surgical treatment of the AAA was undertaken and a few months post-surgery, tryptase levels dramatically decreased to 11.6 ng/mL in P1 and to 14.5 ng/mL in P2. P1 is now in good health and has been undergoing VIT for 3 years without adverse reactions; during VIT he has been stung by hymenoptera without complaining any reaction. P2 is in good health and has been directed to undergo VIT, refusing it for practical reasons. **Table 1** shows demographic and clinical characteristics.

These two patients show that in some cases elevated basal tryptase levels may be related to AAAs inasmuch as post-surgery tryptase

Table 1 *- Demographic, clinical and diagnostic characteristics of P1 and P2.*

		P 1	**P 2**
Age (y) /Sex		67/M	77/M
Allergic reaction (Muller grade)		III	III
Polistes species sIgE (kU/L)		0.34	0.31
Vespula species sIgE (kU/L)		2.81	2.10
Vespa crabro sIgE (kU/L)		0.89	3.59
Basal serum tryptase (ng/mL)		52.8	153
Final diagnosis		Vespula venom anaphylaxis	Vespa crabro venom anaphylaxis
BM results	Multi-focal, dense mast cell infiltration (> 15 mast cells in aggregates) in samples of BM and/or in extracutaneous organs	Negative	Negative
	Presence of D816V KIT mutation in BM, blood, or extracutaneous tissues	Negative	Negative
	Baseline serum tryptase concentration of > 20 ng/mL	Positive	Positive
	Expression of KIT plus CD2 and/or CD25 in mast cells from BM	Negative	Positive
	Mast cells with atypical or spindle shape > 25%	Negative	Negative
MRI		Abdominal aneurysm (diameter = 58mm)	Abdominal aneurysm (diameter = 54mm)
VIT		Vespula species	Not performed
Post surgery tryptase level (ng/mL)		11.6	14.5

SPT: skin prick test; ID: intradermal test; BM: bone marrow; MRI: magnetic resonance imaging; VIT: venom immunotherapy.

levels decreased significantly. Interestingly, even though the patients did not fulfil the criteria for SM diagnosis, P2 had a 0.15% of mast cells expressing CD2/CD25 antigens. This finding, in the light of the high serum tryptase level, could suggest the presence of a mast cell clonal disorder as recently described in HV allergy. Anyway, the real origin of this phenomenon is unknown and we can only hypothesize that it represents the molecular basis of the mast cells invasion with tryptase release of the arterial wall, leading to AAA. As a consequence, the tryptase liberated from the aneurysmatic lesion could act as a risk factor for HV anaphylaxis in patients with a HV sensitization. Otherwise, one might hypothesize that in patients with HV anaphylaxis the high tryptase levels could alter the aortic wall on a preexisting lesion, facilitating the development of an AAA. Moreover, it is known that the role of mast cells in the pathogenesis of AAA formation consists in the degradation of extracellular matrix, apoptosis of smooth muscle cells, activity of the renin-angiotensin system and neovascularization. (15). The follow-up of our patients at 2 years after surgery showed stable low-range tryptase values and no AAA relapse, thus further confirming the probable relationship between the two events. Only future studies on larger study populations will allow to better address this issue.

In conclusion, the marked increase in basal tryptase levels in subjects with a history of HV anaphylaxis, may be associated with undiagnosed aneurysmatic disease. Being that the high levels of tryptase in patients with HV anaphylaxis occur in subjects who remain otherwise asymptomatic, on the light of the present observations we believe that in depth studies may give insight as to all the circumstances that can determine an accumulation of mastocytes that in turn give way to an increase in the reactivity of the HV allergic subjects.

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