Tryptase increase on the first day of hymenoptera venom immunotherapy might be a predictor of future systemic reactions during treatment

Short tittle: Tryptase predicts VIT future tolerance

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ABSTRACT

Background: Serum tryptase (ST) decreases in long-term venom immunotherapy (VIT). A circadian tryptase variation with a small decrease has been found after sting challenge. Both findings have been related to successful VIT.

Objective: To assess whether variation (increase or decrease) in ST on the first day of VIT is associated with the likehood of future systemic adverse reactions (SAR) during treatment.

Methods: We prospectively studied patients who underwent cluster VIT and continued it for at least 6 months. ST was measured on the first day of VIT, before the first dose (pre-IT tryptase) and after the last dose (post-IT tryptase). Differences between patients' groups (with and without SAR) were analysed.

Results: One hundred and sixty VIT were administered to 150 patients. The median baseline ST value was 4.3 µg/L. A total of 25 VIT (15.6%) were associated with SAR. In 64% of the 25 patients with SAR, post-IT tryptase value was higher than pre-IT tryptase level; the median increment was 19% in these patients. We found a significant relation between this increase in tryptase level on the first day of VIT and future SAR (risk ratio 7.6). This elevation was independent of the VIT scheduled day and severity of the SAR, as well as of the basal ST value.

Conclusions: A slight increase in tryptase on the first day of VIT is an independent variable strongly related to a high risk of future SAR. This simple biomarker could be helpful to improve patients' safety.

Key words: Venom immunotherapy, Tryptase, Hymenoptera allergy, Immunotherapy adverse reactions, Systemic mastocytosis.

RESUMEN

Antecedentes: Se ha observado una disminución progresiva del nivel de triptasa sérica (TS) basal durante la inmunoterapia con veneno de himenópteros (ITVH), así como la conservación de la variación circadiana de triptasa en pacientes que han tolerado una repicadura controlada. Ambos hallazgos se han relacionado con la eficacia del tratamiento.

Objetivo: Estudiar si la variación (aumento o disminución) de la TS durante el primer día de ITVH se relaciona con un mayor riesgo de presentar reacciones adversas sistémicas (RAS) con futuras dosis de ITVH.

Método: Estudio prospectivo de pacientes sometidos a ITVH en pauta de inicio agrupada y que continuaron con el tratamiento durante al menos 6 meses. Se determinó la TS el primer día de ITVH, antes de la primera dosis (triptasa pre-IT) y tras la última dosis (triptasa post-IT). Se analizaron las diferencias entre los dos grupos de pacientes (con o sin RAS).

Resultados: Se administraron 160 ITVH a 150 pacientes. El valor medio de TS basal fue 4,3 µg/L, siendo > 11,4 µg/L en 4 casos. Un total de 25 ITVH (15,6%) presentaron RAS. En 64% de los 25 pacientes con RAS, el valor de triptasa post-IT fue más alto que el valor de triptasa pre-IT; el incremento medio fue del 19% en estos pacientes. Encontramos una relación significativa entre este aumento de triptasa el primer día de ITVH y la aparición de RAS con futuras dosis de ITVH (risk ratio 7,6). Esta elevación fue independiente del día de aparición de la reacción, de la gravedad de la misma, así como del valor basal de triptasa.

Conclusiones: Un ligero aumento de triptasa el primer día de ITVH es una variable independiente, fuertemente relacionada con un alto riesgo de presentar una futura RAS. Este sencillo biomarcador podría ser útil para mejorar la seguridad de estos pacientes.

Palabras clave: Inmunoterapia con veneno de himenópteros, Triptasa, Alergia a Himenópteros, Reacciones adversas con Inmunoterapia, Mastocitosis sistémica.

INTRODUCTION

Tryptase is a chemical mediator produced and secreted by mast cells. Baseline serum tryptase (ST) level correlates with body mast cell load and, in acute allergic reactions, it provides a measure of mast cell activation [1]. Several authors have reported an association of Hymenoptera venom allergy with elevated baseline ST: higher levels have been associated with the presence of systemic mastocytosis (SM) or clonal mast cell activation disorders (cMCAS) [2], a risk of severe hymenoptera sting reactions [3,4] a lower efficacy of venom immunotherapy (VIT) [3] and an increased incidence of adverse reactions to VIT [5].

It has been reported that the baseline ST level in patients receiving VIT decreases by 2.5% per year [6], though the natural tendency in the healthy population is a slight increase with age. At the same time, tryptase concentration seems to exhibit a circadian variation, with slightly higher values in the morning. In this context, a temporary increase in ST in patients who developed systemic reactions after sting challenge has been reported [7]. Thus, the presence of this slight decrease in baseline ST concentration, both long and short term, has been associated with successful treatment.

Subcutaneous VIT has demonstrated very high efficacy, with a rate of protection against new stings induced anaphylaxis in around 85-98% of people treated [8]. Despite this efficacy, systemic adverse reactions (SAR) can frequently develop. The rate of SAR with VIT is between 5 and 40% [9,10] with one-fifth of these reactions being severe. The major risk factor associated with SAR is honeybee venom immunotherapy [11,12]. A variety of other factors, potentially related to an increased risk of SAR have been reported, such as

female gender [11], ultrarush protocols [11,12], antihypertensive therapy [12], high basophil allergen sensitivity, Api m 4 sensitization [13], and a short interval between insect sting an onset of symptoms [14].

This study was designed to assess the existence of a possible relationship between variation in ST levels in response to VIT initial doses and the development of future SAR during long-term VIT.

MATERIAL AND METHOD

This was a prospective study carried out in the Allergy Section of the University Hospital of Guadalajara, Spain. The period of analysis was from January 2008 to April 2016. The study was approved by the hospital Ethics Committee.

Patients

Patients of any age diagnosed with Hymenoptera venom allergy who agreed to start VIT and continue receiving it for at least 6 months were included. Patients, who dropped out before month 6 of VIT without having suffered a SAR were excluded.

Hymenoptera venom allergy diagnosis was made according to the recommendations of the European Academy of Allergy and Clinical Immunology [15] maintained in the recent guideline [16], including a detailed clinical history and a complete allergic workup performed at least 4 weeks after the sting reaction, which comprised skin prick and intradermal tests for the main Hymenoptera venoms: *Apis mellifera*, *Vespula spp* and *Polistes dominula* (ALK-Abelló, Madrid, Spain); baseline ST (ImmunoCAP tryptase, Thermo Fisher, Uppsala, Sweden); total IgE (Immulite® 2000, Siemens Diagnostics, Germany); and specific IgE to *Apis, Vespula* and *Polistes dominula* (ImmunoCAP, Thermo

Fisher, Uppsala, Sweden). Patients' clinical and demographic characteristics were collected.

In patients with suspected mast cell disease, a complete bone marrow study was performed to diagnose SM according to the current World Health Organization (WHO) criteria [17], and cMCAS according to published proposals [18,2,19]. The REMA score was used as a screening tool for suspecting the presence of cMCAS in our patients [19].

Venom Immunotherapy (VIT)

VIT was administered following the recommendations of the European Academy of Allergy and Clinical Immunology subcommittee on insect venom allergy [9] using *Apis mellifera*, *Vespula spp* or *Polistes dominula* venom (Pharmagen ALK-Abelló, Madrid, Spain or Allbey Stallergenes, Paris, France) according to individual sensitization profile. Patients received subcutaneous VIT using a build-up cluster schedule [20] as shown in Figure 1, with administration of several doses each day, within a 30-minute interval, on 2 non-consecutive days, until a 100µg dose was achieved. Then, this maintenance dose was administered monthly during the first year, and at increasing time intervals to every 6-8 weeks subsequently until 5 years of treatment were completed. Patients were not pre-treated with antihistamines before VIT doses.

All VIT doses were administered in a hospital setting, with personnel trained in early recognition and treatment of adverse reactions. On the day of VIT administration, doses and adverse reactions were recorded. SAR were graded in accordance with the World Allergy Organization (WAO) classification as reported by Cox et al. [21]. Management of SAR also followed established WAO recommendations [22]. In patients who experienced a SAR, pre-treatment with antihistamines (alone, or combined with oral corticosteroids) was used before subsequent doses.

Measurement of serum tryptase

Blood samples were obtained on the first day of VIT to measure tryptase before the first dose (pre-IT tryptase) and 90-120 minutes after the last dose (post-IT tryptase). Both serum samples

were stored at 4ºC when measurements were performed on the same day, or at -20ºC if the analysis was done another day. The time interval between the two blood draws was 3–3.5 hours (first blood sample at 8:30–9:00 am hours, and the second at 11:30–12:00 am hours). Both serum samples were analysed simultaneously in the same assay (ImmunoCAP tryptase, Thermo Fisher, Uppsala, Sweden). SARs and their association with patients' baseline tryptase level, pre-IT tryptase and post-IT tryptase were evaluated. Tryptase concentrations exceeding 11.4 µg/L (95^{th} percentile for healthy non-allergic individuals, as determined by the manufacturer) were considered elevated. The interassay variability of tryptase technique is estimated as $\leq$ 5% (manufacturer information).

Statistical analysis

Quantitative variables are presented as medians with corresponding interquartile ranges (IQRs). Categorical variables are shown as percentages. Bivariate analyses were performed using the χ^2 test and the non-parametric Mann-Whitney U test for assessing differences between groups with and without SAR. *P* values less than 0.05 were considered significant. When significant differences were found the risk ratio (RR) was estimated. All statistical analyses were performed in SPSS 20.0 (SPSS Inc., Chicago, IL).

RESULTS

Sample profile

Patients

A total number of 155 patients started VIT. Five patients were excluded: 2 of them dropped out before month 6 of VIT without having suffered a SAR and the other 3 had received VIT for less than 6 months at the end of the study. One hundred and fifty patients were included (106 men and 44 women, with a median age of 45 years (IQR 35 – 55.7). Nine patients were diagnosed with SM

or cMCAS (6%; 95% CI, 2.2–9.8%). Clinical and demographic data are summarized in Table 1.

Basal tryptase

The median baseline tryptase value of the sample was 4.3 µg/L (IQR 3.1 – 5.4 µg/L). In 4 patients the tryptase levels were pathologically high (> 11.4 µg/L), being only in one of them higher than 20µg/L (minor diagnostic criteria for mastocytosis).

Insect sting

Bees were the hymenoptera species most frequently associated with sting reactions (43%), due to the great implementation of beekeeping in our area.

Venom Immunotherapy

A hundred and sixty VITs were administered. Ten patients received double VIT: in 8 double-sensitized patients because it was not possible to identify the culprit insect (*Polistes* and *Vespula*), and in two patients who developed systemic allergic reactions with two different hymenoptera stings (*Apis* and *Polistes*).

Tryptase was measured on the first day of VIT in 158 treatments; levels decreased after the 4 doses of VIT (post-IT tryptase), compared to the pre-IT tryptase value, in 87% of the treatments. The average percent decline was 11.2%.

Systemic adverse reactions to immunotherapy

A total of 56 systemic adverse reactions were recorded in 25 VIT (15.6% of total VIT) administered to 25 patients (21 men and 4 women, with a median age of

46.7 years). Fourteen out of 25 patients suffered a single SAR. Most of these patients (76%) were receiving bee VIT compared to 37% of bee VIT in the no-SAR group ($P < 0.001$).

Severity and timing of reaction

Half of all SAR were grade 1 (52%) and did not require treatment in 45% of them. Severe reactions (grades 3 and 4) accounted for 21.4% of all SAR (10 patients; 6.25% of all VIT). SAR onset occurred during the build-up phase in 18 (72%) of the 25 patients. Considering the 56 reactions, 46.4% took place during the escalating doses and 53.6% during the maintenance doses (Table 2), in 18 and 12 patients respectively. Five patients developed SAR during both build-up and maintenance phases. Ten patients suffered SAR on the first day of VIT: 9 experienced a single SAR and one patient 2 reactions (cutaneous pruritus with 2 doses). Nine of these SAR were grade 1, as shown in Table 2.

Clonal mast cell activation syndrome

Three patients diagnosed with cMCAS or SM received bee VIT and 6 received vespid VIT (one patient was treated with both *Vespula* and *Polistes* VIT). All three bee venom allergic patients developed SAR: two had severe grade 4 reactions, and one had a mild reaction (facial flushing). Only one out of 6 vespid VIT in patients with cMCAS elicited a SAR (14%). Altogether, 4 out of 10 VITs (40%) administered to the 9 patients with cMCAS caused SAR compared to 14% of VITs in 141 patients with no cMCAS ($P = 0.02$). All patients but one were diagnosed with SM or cMCAS after the hymenoptera sting allergic reaction.

Tryptase

There was no significant association between SAR and baseline tryptase levels (median tryptase was 4.2µg/L in the SAR group versus 4.7µg/L in the no-SAR group; *P* = 0.24). We found no differences using a cut-off point of 5 µg/L (40.8% in the SAR group, vs. 33.8% in the no-SAR group, *P* = 0.23) or using a cut-off point of 11.4 µg/L (1.5% in the SAR group, 8% in the no-SAR group, *P* = 0.055). However, in 16 out of 25 patients (64%; 95% CI, 45.2–82.8%) who developed SAR, the post-IT tryptase value on the first day of VIT was higher than the pre-IT tryptase (Figure 2). Median tryptase increase was 19% in these 16 patients, but only 5 of them developed a SAR on that day, and 4 out of these 5 patients had a grade 1 reaction. We found a significant association between a slight increase in tryptase level on the first day of VIT and the development of future SAR (RR 7.6; 95% CI 3.7–15.5; *P* < 0.001) (Table 3). Such increase was independent of both the VIT scheduled day of onset and the severity of the SAR, as well as of the basal ST value. Conversely, in patients who did not develop SAR, a mean 12% decrease in ST concentration was found when comparing post-IT and pre-IT tryptase values. In fact, when post-IT tryptase values were higher than pre-IT values, the average rate of SAR was 53%, versus only 7% in patients whose post-IT tryptase values were unchanged or lower than pre-IT values (*P* < 0.001). There were no significant differences in the tryptase behaviour between patients with and without cMCAS.

Venom

Analysis of different venom types revealed a higher risk of SAR in patients receiving bee VIT (RR 7.8, 95% CI 3.58–17) compared to those receiving vespids IT (RR 0.42, 95% CI 0.18–0.99) (*P* < 0.001).

DISCUSSION

In this study we report an analysis of tryptase variation on the first day of VIT and its association with SAR during VIT treatment. It is well known that serum tryptase is a good marker of individual mast cell load, or mast cell activation at one point in time [1]. The current standard assay ~~to detect~~ for ST measures both inactive tryptases (Pro-α/β-tryptases which reflect genetic factors and individual mast cell load) and mature active β-Tryptase, (which is stored in mast cell granules and released upon mast cell activation) [23,24]. Baseline tryptase value was not related with SAR in our patients, even when different cut-off points were considered; there were no differences when using a cut-off point of 5 µg/L (P = 0.23) or a cut-off point at the upper limit of normal, 11.4 µg/L (P = 0.055). Several studies have associated elevated baseline ST levels with the occurrence or likelihood of SM [25,26], a higher risk of severe sting reactions [3,5], and an increase of SAR to VIT [27]. Low levels have been associated with alcohol consumption [27,28]. Elevated ST ~~is~~ levels have been reported in 7-11% of patients with Hymenoptera venom allergy [2,4]. In our sample only four patients had an ST level >11.4 µg/L (2.6% of all VITs); this rate is clearly lower than those described by other authors. Only in one patient ST was above 20 µg/L, value considered a minor criterion for mastocytosis by the WHO definition [17]. As the diagnosis of SM and/or cMCAS was proven in 9 patients through a bone marrow biopsy, we can state with certainty that baseline ST level is not a useful predictor of cMCAS in our patient population, as previously described [2,18]. cMCAS patients are, sometimes, early stages of indolent types of SM, with a lower mast cell burden. Consequently baseline ST levels might be normal. Therefore, clinical suspicion is mandatory to diagnose this disease

[18,19]. Long life immunotherapy is recommended for these cMCAS patients [29,30]

Separate analysis of bee and vespids VIT revealed a significant association of SAR related to venom; all patients with cMCAS who were treated with bee VIT experienced SAR, compared to a 14.3% reactor's rate with vespids VIT (P = 0.004). The small number of patients with mastocytosis or cMCAS in our study (6 patients: 3 received bee VIT and 6 vespid VIT) precludes further conclusions, but it seems that SAR should only be expected in patients with SM treated with bee VIT, unlike in previous Central European studies that associated it with vespids VIT [5]. After these results we treat patients with high suspicion of suffering cMCAS with Cromoglicate prior to the onset of VIT, especially when they receive bee VIT.

Though baseline tryptase value was not associated with SAR in our sample, we did find very significant differences with respect to tryptase variations on the first day of VIT. A significant decline in baseline ST of about 2.5% per year during VIT has been reported [6]. Conversely in healthy individuals, ST increases linearly with age by about 1.3% per year [4]. In this study, we found such a decline on the very first day of VIT in 87% of treatments, in which the pre-IT tryptase value was higher than the level recorded after the 4th VIT dose (post-IT tryptase). In our sample, we found a median ST reduction of 12% on the first day of VIT in patients who did not have SAR versus a median rise of 19% in those who developed SAR (P < 0.001). Dugas-Breit et al measured ST at 20 min, 90 min and 18 hours after a sting challenge test in 20 patients [7], and found a significant drop of ST from baseline levels both at 20 min (-18%) and 90 min (-30%) after the sting in patients who developed no systemic reaction, with

a return to baseline levels at 18 hours. The results were similar in not stung controls. The one patient with a mild post-sting systemic reaction showed a slight increase in ST concentration from baseline. The authors concluded that this ST decrease could represent a natural circadian variation with lower levels in the afternoon and higher levels in the morning and suggest that a slight decrease or unchanged ST concentration after challenge are associated with a successful therapy. The decrease of tryptase concentrations on day 1 of VIT in our non-reactors seems to be a reflection of the normal circadian decrease. It must be highlighted that in our study all blood samples were collected in the morning, with a 3 to 3.5-hour time interval between them, while Dugas-Breit et al. collected the first blood sample in the morning (at 9:00 a.m.) and the second in the afternoon (between 2:20 and 3:20 p.m.) In those two previously published works [6,7], as in our study, the decrease in ST was found to be independent of the initial baseline tryptase value.

Regarding underlying mechanisms VIT induces a tolerant state in peripheral T cells, initiated by the actions of IL-10, among others mediators [31]. It has been postulated that IL-10 may also decrease mast cell density and growth, and might thus reduce baseline ST during VIT [32,33]. Though an increment in IL-10 levels 24 hours after VIT initiation has been described [34], the exact timing of onset of these changes is not known; it seems speculative to think that IL-10 can play a role in this early tryptase decline.

It seems that these variations actually could be a consequence of circadian changes, and it has been clearly demonstrated in our study, as in previous investigations, that this tryptase behaviour seems to occur both in the short and

long term, and this finding is desirable in patients receiving VIT, as a reflection of good tolerance to treatment and, in previous studies, of VIT efficacy.

We have observed that this downward trend is reversed in most patients who develop SAR during VIT regardless of the day of SAR development, even with grade 1 reactions. In anaphylaxis, the proportion of elevated tryptase values has been described as low in grade 1 (0%) and 2 (4%) reactions [35]. As a matter of fact, in our patients, a pre-IT tryptase < post-IT tryptase value was the major risk factor for SAR (RR 7.6; 95% CI 3.7–15.5; $P < 0.001$). The risk ratio increases to 7.8 in bee venom allergic patients (95% CI 3.58 - 17; $P < 0.001$).

It would be of interest to know the exact behaviour of β-tryptase, as well as other mast cell markers, such as chimase and carboxypeptidase. Perhaps this might clarify the pathophysiological mechanisms responsible for these findings.

CONCLUSIONS

We conclude that an increase in tryptase levels on the first day of VIT is an independent variable strongly associated with a high risk of future SAR. It is independent of the VIT scheduled day of the SAR, the severity of the reaction, and the baseline tryptase value.

The use of tryptase variation in response to VIT is a simple biomarker that could be helpful to improve patients' safety.

The presence of SM or cMCAS is a risk factor for developing SAR with VIT, and was related to bee VIT in our patients.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

PREVIOUS PRESENTATION

The data included in this manuscript were presented in poster format during the European Academy of Allergy and Clinical Immunology (EAACI) Annual Congress in Helsinki, Finland, June 2017.

REFERENCES

1. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicador of mast-cell activation in systemic anaphylaxis and mastocytosis. N Engl J Med 1987;316:1622-6.
2. Bonadonna P, Perbellini O, Passalacqua G, Caruso B, Colarossi S, Dal FD, et al. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. J Allergy Clin Immunol 2009;123:680-686
3. Haeberli G, Bronnimann M, Hunziker T, Muller U. Elevated serum tryptase and Hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. Clin Exp Allergy 2003; 33:1216-20.
4. Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitkowski M. Basal serum tryptase level correlates with severity of hymenoptera sting and age. Allergy 2010;65:919-923.
5. Ruëff F, Przybilla B, Biló MB, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase-a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. J Allergy Clin Immunol. 2009;124(5):1047-54.
6. Dugas-Breit S, Przybilla B, Dugas M, Arnold A, Pfundstein G, Küchenhoff H, Ruëff F. Serum concentration of baseline mast cell tryptase: evidence for a decline during long-term immunotherapy for Hymenoptera venom allergy. Clin Exp Allergy 2010;40:643-649.
7. Dugas-Breit S, Przybilla B, Schöpf P, Ruëff F. Possible circadian variation of serum mast cell tryptase concentration. Allergy 2005;60:689-692.
8. Golden DBK. Insect sting allergy and venom immunotherapy: A model and a mystery. J Allergy Clin Immmunol 2005; 115: 439-47.
9. Muller U, Mosbech H, editors. Position paper. Immunotherapy with Hymenoptera venoms. Allergy 1993; 48 (Suppl 14): 37-46
10. Bonifazi F, Jutel M, Bilo BM, Birnbaum J, Muller U. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy. 2005;60(12):1459-70.

11. Mosbech H, Müller U. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. European Academy of Allergology and Clinical Immunology. Allergy 2000;55(11):1005-10.

12. Ruëff F, Przybilla B, Biló MB, Müller U, Scheipl F, Aberer W, et al. Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: the importance of baseline serum tryptase. J Allergy Clin Immunol. 2010;126(1): 105–11.

13. Ruiz B, Serrano P, Moreno C. IgE-Api m4 is useful to identify a particular phenotype of allergy to bee venom. J Investig Allergol Clin Immunol 2016; 26(6) doi: 10.18176/jiaci.0053.

14. Korosec P, Ziberna K, Silar M, Dezman M, Celesnik Smodis N, Rijavec M, Kopac P, Erzen R, Lalek N, Barjovic N, Kosnik M, Zidarn M. Immunological and clinical factors associated with adverse sysstemic reactions during the build-up phase of honeybee venom immunotherapy. Clin Exp Allergy 2015;45:1579-1589.

15. Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN; EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of Hymenoptera venom allergy. Allergy 2005;60(11):1339-49.

16. Sturm GJ, Varga E-M, Roberts G, Mosbech H, Bilò MB, Akdis CA, Antolín-Amérigo D, Cichocka-Jarosz E, Gawlik R, Jakob T, Kosnik M, Lange J, Mingomataj E, Mitsias DI, Ollert M, Oude Elberink JNG, Pfaar O, Pitsios C, Pravettoni V, Ruëff F, Sin BA, Agache I, Angier E, Arasi S, Calderón MA, Fernandez-Rivas M, Halken S, Jutel M, Lau S, Pajno GB, van Ree R, Ryan D, Spranger O, van Wijk RG, Dhami S, Zaman H, Sheikh A, Muraro A. EAACI guidelines on allergen immunotherapy: Hymenoptera venom allergy. Allergy. 2017;00:1–20.

17. Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB, Marone G, Nuñez R, Akis C, Sotlar K, Sperr WR, Wolff K, Brunning RD, Parwaresch RM, Austen KF, Lennert K, Metcalfe DD, Vardiman JW, Bennet JM. Diagnostic criteria and classification of mastocytosis; a consensus proposal. Leukocyte Res 2001; 25:603-25.

18. Sonneck K, Florian S, Müllauer L, Wimazal F, Födinger M, Sperr WR, et al. Diagnostic and subdiagnostic accumulation of mast cells in the bone marrow of patients with anaphylaxis: monoclonal mast cell activation syndrome. Int Arch Allergy Immunol 2007;142:158-64.
19. Alvarez-Twose I, González de Olano D, Sánchez-Muñoz L, Matito A, Esteban-López MI, Vega A, Mateo MB, Alonso Díaz de Durana MD, de la Hoz B, Del Pozo Gil MD, Caballero T, Rosado A, Sánchez Matas I, Teodósio C, Jara-Acevedo M, Mollejo M, García-Montero A, Orfao A, Escribano L. Clinical, biological, and molecular characteristics of clonal mast cell disorders presenting with systemic mast cell activation symptoms. J Allergy Clin Immunol. 2010 Jun;125(6):1269-1278
20. Sánchez-Machín I, Moreno C, González R, Iglesias-Souto J, Pérez E, Matheu V. Safety of a 2-visit cluster schedule of venom immunotherapy in outpatients at risk of life-threatening anaphylaxis. J Investig Allergol Clin Immunol 2010;20(1):91-2.
21. Cox L, Larenas-Linnemann D, Lockey RF, Passalacqua G, editors. Speaking the same language: the World Allegy Organization subcutaneous immunotherapy systemic reaction grading system. J Allergy Clin Immunol 2010;125:569-74.
22. Simons FER, Ardusso LRF, Bilò MB, El-Gamal YM, Ledford DK, Ring J, Sanchez-Borges M, Senna GE, Sheikh A, Thong BY. World Allergy Organization anaphylaxis guidelines: Summary. J Allergy Clin Immunol 2011;127:587-93.
23. Schwartz LB, Bradford TR, Rouse C et al. Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. J Allergy Clin Immunol 1994; 14: 190-204.
24. Müller U. Elevated baseline serum tryptase, mastocytosis and anaphylaxis. Clin Exp Allergy. 2009; 39: 620-622.
25. González-de-Olano D, Alvarez-Twose I, Vega A, Orfao A, Escribano L. Venom immunotherapy in patients with mastocytosis and hymenoptera venom anaphylaxis. Immunotherapy. 2011 May;3(5):637-51.
26. Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB, Marone G, Nuñez R, Akis C, Sotlar K, Sperr WR, Wolff K, Brunning RD, Parwaresch RM, Austen KF, Lennert K, Metcalfe DD, Vardiman JW, Bennet JM. Diagnostic criteria and classification of mastocytosis; a consensus proposal. Leukocyte Res 2001; 25:603-25.

27. Carballada F, Alonso M, Vizcaino L, Coutinho V, Núñez R, Vidal C, Boquete M, González-Quintela A. Serum tryptase concentrations in beekeepers with and without hymenoptera venom allergy. J Invest Allergol Clin Immunol 2013;23:30-36.

28. Beceiro C, Campos J, Valcarcel MA, Fenger RV, Lojo S, Linnemberg A, Vidal C, Gonzalez-Quintela A. Serum concentrations of mast cell tryptase are reduced in heavy drinkers. Alcohol Clin Exp Res 2015;39:672-8.

29. Bonadonna P, Lombardo C, Zanotti R. Mastocytosis and allergic diseases. J Investig Allergol Clin Immunol. 2014;24(5):288-97.

30. Alfaya Arias T, Soriano Gómis V, Soto Mera T, Vega Castro A, Vega Gutiérrez JM, Alonso Llamazares A, Antolín Amérigo D, Carballada Gonzalez FJ, Dominguez Noche C, Gutierrez Fernandez D, Marques Amat L, Martinez Arcediano A, Martinez San Ireneo M, Moreno Ancillo A, Puente Crespo Y, Ruiz Leon B, Sánchez Morillas L; Hymenoptera Allergy Committee of the SEAIC. Key Issues in Hymenoptera Venom Allergy: An Update. J Investig Allergol Clin Immunol. 2017;27(1):19-31. doi:10.18176/jiaci.0123.

31. Akdis CA, Blesken T, Akdis M, Wuetrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest 1998; 102: 98-106.

32. Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory cells. J Allergy Clin Immunol 2009;123:735-46.

33. Speiran K, Bailey DP, Fernando J, Macey M, Barnstein B, Kolawole M, Curley D, Watowich SS, Murray PJ, Oskeritzian C, Ryan JJ. Endogenous supresión of mast cell development and survival by IL-4 adn IL-10. J Leukocyte Biol 2009;85:826-36.

34. Riccio AM, Saverino D, Pesce G, Rogkakou A, Severino M, Bonadonna P et al. Effects of different up-dosing regimens for hymenoptera venom immunotherapy on serum CTLA-4 and IL-10. PLoS One. 2012;7(6):e37980.

35. Vadas P, Perelman B, Liss G. Platelet-activating factor, histamine and tryptase levels in human anaphylaxis. J Allergy Clin Immunol 2013;131:144-149.

TABLES AND FIGURES

Table 1. Clinical and demographic data for the total of patients, the total VIT administered, and the subgroups with and without systemic adverse reactions (SAR)

	Patients	**Total VIT**	**No SAR**	**SAR**	***P***
N (%)	150	160	135 (84.4)	25 (15.6)	
Age Median (IQR)	45 (35-55.7)	45.5 (35.5-56)	45 (35-55)	46 (40.5-58)	0.39
Gender M/F	106 / 44	112 / 48	91 / 44	21 / 4	0.88
cMCAS N(%)	9 (6)	10 (6.3)	6 (4.4)	4 (16)	0.02
Tryptase µg/L (IQR)	4.3 (3.1–5.4)	4.2 (3.1–5.4)	4.2 (3.1–5.4)	4.7 (3.1–6.3)	0.24
Total IgE UI/mL (IQR)	95.9 (38–224.5)	102 (38–231)	109.5 (43–253)	73 (32–143)	0.1
Müller grades N (%)*****					
I	47 (31)	49 (31)	43 (32)	7 (28)	
II	24 (16)	24 (15)	23 (17)	1 (4)	0.42
III	53 (35)	58 (36)	47 (35)	11 (44)	
IV	28 (18)	28 (18)	22 (16)	6 (24)	
Venom N (%)					
Apis		69 (43.1)	50 (37)	19 (76)	0.001
Polistes		49 (30.6)	45 (33.3)	4 (16)	
Vespula		42 (26.3)	40 (29.6)	2 (8)	
Specific IgE kU/L (IQR)		7 (2-19.5)	6.6 (2–22)	8.5 (2.8–15)	0.69

Pre-IT tryptase µg/L (IQR)	4.1 (3–5.5)	4.1 (3–5.4)	4.5 (2.8–6)	0.5
Post-IT tryptase µg/L (IQR)	3.7 (2.8–5.2)	3.6 (2.8–4.9)	5 (2.5–7.1)	0.12
Tryptase behaviour % up	13.3	10.5	64	< 0.001

VIT: Venom immunotherapy; SAR: systemic adverse reactions; *P* value comparing No SAR and SAR groups; N: number; IQR: interquartile range; M/F: male / female ratio; cMCAS: clonal mast cell activation syndrome; Pre-IT tryptase: tryptase value on the first day of VIT, before administration of the first dose; Post-IT tryptase : tryptase value after administration of the last dose on the first day of VIT; Tryptase behaviour % up: percentage of VIT with a post-IT tryptase value higher than pre-IT tryptase value.
***** Two patients developed allergic reactions with two different hymenoptera stings; Müller grade was different depending on the culprit insect.

Table 2. Systemic adverse reactions: VIT scheduled day of onset and severity grade of the 56 reactions

Systemic adverse reactions graded in accordance with the Cox et al. World Allergy Organization

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Total (%)	29 (51.8%)	15 (26.8%)	5 (8.9%)	7 (12.5%)	0 (0%)
Build-up doses					
Day 1	9	1	1	-	-
Day 8	9	-	2	4	-
Maintenance doses					
Day 22	-	-	-	3	-
Subsequent doses	11	14	2	-	-

classification [21].

Table 3. Variables and their association with systemic adverse reactions during VIT

Variable	Total No	No SAR %	SAR %	RR	95% CI	*P*
Bee venom	69	37	76	4.17	1.76–9.89	0.001
cMCAS	9	4.4	16	2.85	1.21–6.72	0.02
*Baseline Tryptase > 11.4	4	1.5	8	-	-	0.055
Tryptase increases	21	10.5	64	7.59	3.72–15.48	< 0.001

SAR: Systemic adverse reactions; RR: risk ratio; CI: confidence interval; cMCAS: clonal mast cell activation syndrome; Tryptase increases: Tryptase value on the first day of VIT after the last dose is higher than the value prior to the first dose.
*RR was not calculated because there was not a significant association (P > 0.05) probably as a result of the small number of patients with this variable.

Figure 1. VIT cluster build-up schedule

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	Venom dose (µg)
Day 1	5 10 20 20
Day 8	50 50
Day 22	100

Days 1 and 8 doses were administered at 30 minutes interval.

Figure 2. Median pre and post-IT tryptase values on the first day of VIT in patients with and without systemic adverse reactions

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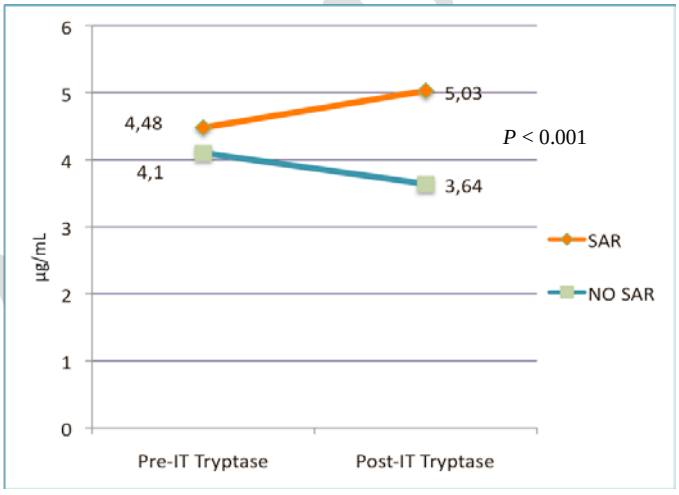
Figure 2: Median pre and post-IT tryptase values on the first day of VIT in patients with and without systemic adverse reactions



SAR: Systemic adverse reactions; Pre-IT tryptase: tryptase value on the first day of VIT, before administration of the first dose; Post-IT tryptase : tryptase value after administration of the last dose on the first day of VIT.
A total number of 160 VIT were included: 25 with SAR, 135 with no SAR.