Clinical and laboratory profile of patients with anaphylaxis to fire ant venom (Solenopsis sp) under specific subcutaneous immunotherapy

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Abstract

Background: Anaphylaxis to fire ant venoms (Solenopsis sp) is a significant cause of systemic reaction caused by Hymenoptera stings in children. There are only a few reports about the safety and efficacy of specific immunotherapy. Objective: Evaluate clinical characteristics, IgE and IgG4 specific responses of patients undergoing immunotherapy with a whole-body extract of Solenopsis sp after one year of the maintenance phase. Materials and methods: Thirty-three patients were enrolled due to anaphylaxis by fire ant venom (Solenopsis sp) and underwent specific immunotherapy. They were assessed at baseline and one year after the beginning of the maintenance phase for skin test; specific venom IgE and IgG4 antibodies; tryptase. Results: All patients included presented a severe anaphylactic reaction. Although two patients (6.25%) presented a tryptase level higher than 11.4 ug/ml, systemic mastocytosis was ruled out. There was no relationship between the severity of the reaction with gender, tryptase level, atopy, previous reactions, the allergen's concentration in the skin test or specific IgE level. There was an increase in the specific IgG4/IgE ratio between the two time points. Reactions were local, with only two mild systemic reactions during the build-up phase. Twenty patients had accidental stings during immunotherapy, with 3 presenting only urticaria. Conclusions: This study is unprecedented in evaluating clinical and laboratory data in the fire ant immunotherapy. Our results show that after one year of the maintenance phase, patients did not develop any severe reaction with only a few mild reactions and presented a significant production of specific IgG4.

Clinical and laboratory profile of patients with anaphylaxis to fire ant venom (*Solenopsis sp*) under specific subcutaneous immunotherapy

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Abstract

Background: Anaphylaxis to fire ant venoms (*Solenopsis sp*) is a significant cause of systemic allergic reaction caused by*Hymenoptera* stings in children. There are only a few reports about the safety and efficacy of specific immunotherapy.

Objective: Evaluate clinical characteristics, IgE and IgG4 specific responses of patients undergoing immunotherapy with a whole-body extract of *Solenopsis sp* after one year of the maintenance phase.

Materials and methods: Thirty-three patients were enrolled due to anaphylaxis by fire ant venom (*Solenopsis sp*) and underwent specific venom immunotherapy. They were assessed at baseline and one year after the beginning of the maintenance phase for skin test; specific IgE and IgG4 antibodies to fire ant venom; tryptase.

Results: All patients included presented a severe anaphylactic reaction. Although two patients (6.25%) presented a tryptase level higher than 11.4 ug/ml, systemic mastocytosis was ruled out. There was no relationship between the severity of the reaction with gender, tryptase level, atopy, previous reactions, concentration of the allergen in the skin test or specific IgE level. There was an increase of the specific IgG4/IgE ratio between the two time points. Reactions were local, with only two mild systemic reactions during the build-up phase. Twenty patients had accidental stings during immunotherapy, with 3 presenting only urticaria.

Conclusions: This study is unprecedented in evaluating clinical and laboratory data in the fire ant immunotherapy. Our results show that after one year of the maintenance phase, patients did not develop any severe reaction with only a few mild reactions and presented a significant production of specific IgG4.

Abbreviations used:

FA: fire ant(s)

WBE: whole-body extract

VIT: venom immunotherapy

QoL: quality of life

Keywords: fire ant, whole-body extract, Hymenoptera, immunotherapy

Introduction

Bees, wasps, and ants are the Hymenoptera involved in accidents causing allergic manifestations. Bees and wasps' venoms are the most well-characterized and purified commercial extracts are available for testing and treatment in the United States and Europe, while ants are much less studied. They are biodiverse, with 22 recognized subfamilies, approximately 300 genera, and more than 14,000 species. However, only a small number of them have been described to cause allergic reactions in humans[1], including the so-called imported fire ants (*Solenopsis sp* .) present in Brazil.

The clinical manifestations produced by *Hymenoptera* venom can be classified into local, large local, systemic anaphylactic and delayed systemic reactions. The risk of a person being stung in an endemic area is from 30% to 60%[2,3], with a 0.16% to 16% risk of presenting severe systemic reactions[4,5].

The diagnosis of anaphylaxis to ant venom is based on clinical history, testing for specific IgE antibodies to the venom, and skin tests after at least 3 to 4 weeks after the acute event to reduce the likelihood of a false-negative result. These tests not only confirm the diagnosis but also identify the appropriate extract to be used in immunotherapy[6].

Allergen-specific immunotherapy in children is indicated for the treatment of allergic rhinitis, allergic asthma, some foods, and Hymenoptera hypersensitivity[7]. Besides being effective in reducing subsequent systemic reactions in children and adults, this treatment significantly increases the quality of life (QoL) of these patients[8].

The treatment is considered to be safe and effective. The reported efficacy for patients treated with bee venom is 77-84%, and 91-96% for patients who received wasp venom[9,10].

Triplett[11] was the first to report successful immunotherapy with whole-body extracts for fire ant venom allergy in 1973. It is widely used in the USA by allergists[12].

There were no systematic studies on the efficacy of fire ant immunotherapy with whole-body extract until Freeman et al[13] in 1992 used sting challenge to assess efficacy. They demonstrated that immunotherapy with whole-body extracts provided a high degree of protection to most patients.

Safe and effective of cluster, rush and ultra-rush schedules for fire ant immunotherapy have been previously reported. Most of the studies with a limited number of patients and the focus were mainly determined whether prophylactic pretreatment with antihistamines and steroids reduced the systemic reaction rate[14,15,16,17,18,19,20].

Adams at al[16] illustrated the safety and efficacy of repeated fire ant rush immunotherapy in three patients. Beveridge et al[17] describe a cluster imported fire ant schedule in a 66-year-old man.

Tankersley et al[18] evaluated fifty-nine patients with imported fire ant hypersensitivity randomized to placebo or twice-daily terfenadine 60 mg, ranitidine 150 mg, and prednisone 30 mg before rush protocol in a double-blinded study. Sting challenges were performed on 56 patients, and they concluded that rush immunotherapy is safe and efficacious; the rate of mild systemic reactions was low.

Dietrich et al[19] included 37 patients in a 1-day rush protocol. Twenty-two were stung challenged, 21 did not result in any signs or symptoms of a systemic reaction, giving an efficacy rate of preventing systemic reactions of 95.5% for the rush protocol.

Arseneau et al[20] evaluate the safety and efficacy of 1-day rush immunotherapy with fire ant whole-body extract (0.5 mL 1:100 (wt/vol) maintenance injection), with a sting challenge on approximately day 22 in 66 patients.

Brown et al[21] reported that venom immunotherapy with*M pilosula* (jack jumper) was very effective in preventing life-threatening sting anaphylaxis, and it could benefit the population in areas of southeastern Australia where *Myrmecia* ant stings occur.

La Shell et al[23] showed that three-year subcutaneous immunotherapy with whole-body extract is safe; however, specific IgE and IgG4 antibodies or tryptase before and after immunotherapy were not evaluated.

Thus, the objective of this study was to evaluate side effects, efficacy and specific IgE and IgG4 production of patients submitted to subcutaneous fire ant immunotherapy with whole-body extracts of*Solenopsis sp.* during the first year of treatment.

Methods:

Types of participants enrolled in this study:

Patients were attended at the ambulatory of anaphylaxis of the Clinical Immunology and Allergy Service of the Hospital das Clínicas - Faculty of Medicine – Sao Paulo University. They presented a history of a severe anaphylactic reaction (Muller's severity score: grade III or IV[24]) after fire ant sting of the species*Solenopsis sp* , and according to the following inclusion criteria:

* Children or adults: age 5 to 65 year

* Diagnosis of type I hypersensitivity confirmed by positive determination of specific IgE level (*Solenopsis sp* - ImmunoCap(r) i70) and a positive skin test (prick or intradermal test).

* Informed consent form signed by the patient or authorized representatives approved by our institutional review board.

The following items were accepted as exclusion criteria: use of immunomodulatory treatment in the last five years; previous treatment with specific allergen immunotherapy; moderate or severe persistent asthma and FEV1 <80%; pregnancy; illiteracy; psychiatric illness; continuous use of β-blockers; cardiovascular diseases, organ failure, and diseases that increase the risk of side effects of adrenaline and other immunological, autoimmune diseases or malignancy.

Research design:

After fulfilling the inclusion criteria, patients underwent the skin test. Prick test method were carried out with positive (histamine base) and negative (saline) controls and the following concentrations of the whole-body extract of the *Solenopsis* mix (HollisterStier; Spokane, USA) were utilized: 1:1,000,000 wt/vol; 1:100,000 wt/vol; 1:10,000 wt/vol; 1:1000 wt/vol, if needed. If the prick result was negative, the prick test was followed by intradermal testing. The test was considered positive when the wheal diameter was 5 mm or greater with a flare of 11 mm or greater for the prick test and for the intradermal test when the wheal enlargement was above 3 mm from the initial wheal caused by the volume of the injected extract and flare greater than 11 mm[18].

After positivity to the skin test, the starting dose of the immunotherapy was calculated as follows: if the positivity was in the prick test, three concentrations were returned to start the treatment. If this test was positive in the intradermal test, two concentrations were returned. The protocol used for immunotherapy with whole-body extract (*Solenopsis* sp) was the cluster-modified schedule, whose concentrations increased weekly until the maintenance phase, which in this study was considered 1:100wt/vol, volume: 0.5mL. Maintenance doses were subsequently spaced to once every 4 weeks. The approval of the project by the Ethics Committee was signed on January 20, 2016 (Online registration nº: 14174). Figure 1 exemplifies the immunotherapy schedule in the build-up phase used with an initial hypothetical dose:

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Figure 1. Build-up phase of immunotherapy using a hypothetical initial dose. Intervals between injections are of 30 minutes.

Skin testing and immunotherapy were performed with whole-body extract (HollisterStier company, Spokane, USA) that consisted of a*Solenopsis invicta* and *Solenopsis richteri* mix from a 50-mL 1:10 wt/vol stock bottle in 0.4% phenol.

The following clinical characteristics: assessment of gender, atopy, age, urban or rural area, and the following tests were performed at baseline: measurement of total IgE, IgG, IgM, IgA antibodies; measurement of serum IgE antibody for *Solenopsis* mix (i70); tryptase level, specific IgG4 antibody, and skin test.

After one year in the maintenance phase of the treatment, the following parameters were evaluated: specific IgE antibody level (i70), specific IgG4 antibody, adverse reactions to immunotherapy, accidental stings, and which were reactions that occurred in those stings.

Statistics

Categorical data were summarized using percentages and analyzed using Pearson's chi-square tests with Yates' continuity correction, Student's t-test, and ANOVA with Tukey's adjustment or Wilcoxon test, whichever most appropriate. Results were considered statistically significant for P values less than 0.05.

Results

Patients

All 33 patients enrolled in the study had histories of systemic reactions (Muller's severity score: grade III or IV) to fire ant stings and positive skin test reactions to the whole-body extract to*Solenopsis* mix. Table 1

During the treatment, when they were in the maintenance phase, three patients gave up the treatment: 2 of them reported that they had been stung twice and had no reactions, added to the difficulty of transportation. The third patient gave up for fear of losing a job.

The majority of systemic reactions (grade III or IV) to fire ants index field stings resulted from 1 sting.

'Table 1: Demographics and clinical features of the patients:

Age, years: Between 5 to 10 years Between 11 to 20 years Between 21 to 30 years Between 31 to 40 years > 41 years	7 - 5
Urban area	31 p
Male	17 p
Asthma	7 pa
Tryptase level Reaction grade III (mean value) Reaction grade IV (mean value) >11,4ng/mL	3,63
Grading system** Grade III Grade IV	24 p
Index field reactions Grade III Grade IV	1 sti

* Patients were referred to the hematology sector, with systemic mastocytosis ruled out.

**Systemic reactions measured using the Mueller grading system

Possible risk factors associated with the severity of the symptoms:

Gender:

There was no correlation between gender and the severity of the reaction (Pearson's Chi-squared test with Yates' continuity correction; p=0.9151). Table 2

Atopy

Seventeen patients (51.51%) reported a diagnosis of allergic rhinitis and/or allergic asthma. The diagnosis of allergic rhinitis and/or asthma did not prove to be a risk factor for the severity of the reaction, as there was no relationship of this disease in patients who presented a grade IV reaction. (p-value = 0.9233788). Table 2

Tryptase level

Patients with an anaphylactic reaction, grade III severity had a mean basal tryptase level of 3.63 ng/mL, with a standard deviation of 3.06 ng/mL. Patients with grade IV anaphylactic reaction had an average of 4.76 ng / mL, with a standard deviation of 6.98 ng/mL. There was no correlation between serum tryptase level and grade IV severity (Wilcoxon rank with continuity correction; p = 0.4333). Table 2

Previous reactions

Considering only Grade-IV severe previous reactions, for natural history assessment purposes, there was no correlation with any specific reaction: 11.11% had a previous grade-II severity reaction; 22.22%, Grade-IV; 55.56%, local and 11.11%, extensive local. In these patients, there was no correlation between reaction severity in previous stings and progression to a severe reaction in future stings (p = 1.207). Most of the patients reported a local reaction. Table 2

Ant fire IgE level (Immunocap i70®)

Considering the correlation between the severity of the reaction and the measurement of specific IgE levels against fire ant (ImmunoCap i70®), there was no statistical difference between the groups (p = 0.776). Patients with a grade III severity had a mean value of 26.3 kU/L, with a standard deviation: 32.4, and patients with grade IV severity had a mean value of 45.9 kU/L and standard deviation: 49.2. Table 2

Skin test

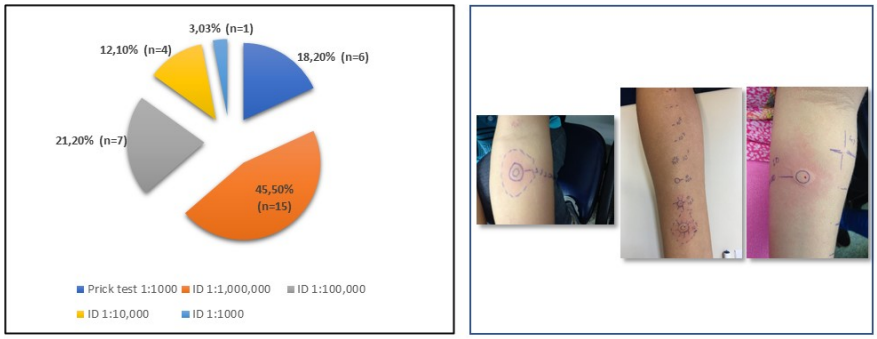
Patients presented the following results in the skin test (prick test and intradermal test), with most of the results being positive in the intradermal test (81.8%), Figure 2.

There was no correlation between concentrations in positive skin test with the severity of the reaction presented (chi-square test; p = 0.7354). Table 2

Figure 2:

(a) Percentage and number of patients (n) of positive skin tests (prick test and intradermal test)

(b) examples of positive skin tests (outpatients Anaphylaxis of HC-FMUSP)



(a) (b)

Table 2: Baseline clinical and laboratory characteristics of the patients

Clinical and laboratory characteristics of the patients	**Grade III**	**Grade IV**	**p-value**
Male Female	12 patients (75%) 11 patients (64,7%)	4 patients (25%) 6 patients (35,3%)	0.9151
Asthma	5 patients (15,15%)	2 patients (6,06%)	0.9233788
Tryptase level (mean value)	3.63 ng/mL	4,76 ng/mL	0.4333
Ant fire IgE level (mean value)	26.3 kU/L	45.9 kU/L	0.776
Skin test Prick test Intradermal test	4 patients (12,12%) 20 patients (60,06%)	2 patients (6,06%) 7 patients (21,21%)	0.7354

Dynamic between specific IgE and IgG4 antibodies

Fire ant IgE antibody level: basal and in the maintenance phase

When evaluating the ratio of the specific IgE level (Immunocap® i70) for each patient before and after one

year in the maintenance phase, there was a statistically significant difference (p= 0.0041). The specific IgE antibody decreased in this interval of time in 73.3% of patients (mean baseline: 31.67 kU/L; after one year in the maintenance phase: 9.86 kU/L, mean).

Serum determination of specific IgG4 antibody

Serum specific IgG4 antibody was performed at baseline and 12 months after the beginning of the maintenance phase (Figure 2a).

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Figure 2. a) Specific IgG4 at baseline and after 12 months of immunotherapy maintenance phase (n = 30). b) Comparison of the ratio specific serum IgG4/IgE levels (baseline and after 12 months in the maintenance phase), (Wilcoxon test, p = 0.0000318).

Specific serum IgG4/IgE ratio

When comparing the serum IgG4/IgE ratio to fire ant venom at baseline and after one year from the beginning of the maintenance phase, there was a statistically significant difference (Wilcoxon test, p = 0.0000318), Figure 2b.

Adverse reactions to immunotherapy

During the build-up phase

During the build-up phase, two patients (6.06%) presented grade-I reaction (only one organ involved limited to skin - mild urticaria), and four patients (36.36%) had local reactions> 50mm after subcutaneous injections. In 57.58%, there was a local reaction smaller than 50mm or no reaction at all.

Considering the risk of reaction in each injection applied, in 1167 applications performed during the build-up phase, with 58.33% of the reactions occurring in dose 1:100wt/vol. The risk of systemic reaction by injection was 0.017% in this phase (Figure 3).

During the maintenance phase:

During the maintenance phase, five patients (15.15%) presented local reactions at the injection site >50mm, and 84.85% presented no reaction or had a reaction less than 50mm. No patient had a systemic reaction in the maintenance phase of the treatment.

When considering the risk of reaction in each injection applied, we had 5 reactions (1,4%) in 360 applications performed during the maintenance phase, being only local reactions (Figure 3).

There was no need to use adrenaline at any time during treatment, and the systemic reactions that occurred in the build-up phase were treated only with antihistamines with improvement after 2 hours in the observation period.

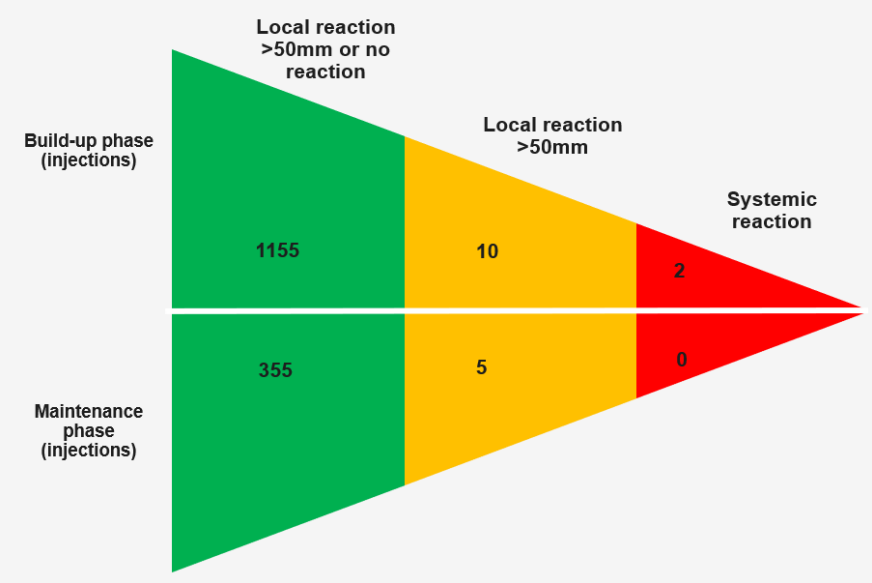

Figure 3: Number of adverse reactions (per injection) during immunotherapy according to phase and reaction type.

Field stings during the treatment

During the treatment, 20 patients (60.6%) presented accidental field stings by fire ants.

During the build-up phase, five patients were stung: four local reactions and one grade-I reaction (mild urticaria) that improved after antihistamine, with no need to seek emergency care or use adrenaline auto-injectable. Figure 4

In total, patients referred nine accidental field stings because they have subsequent stings: 2 patients had experimented stings two times during the build-up phase, one patient was stung three times, and two patients had a single sting.

During one year of observation in the maintenance phase: nineteen patients (57.6%) were accidentally stung, with seventeen patients (89.5%) having experimented a local reaction and two patients (10.5%) having grade-I systemic reactions: urticaria, that improved after taking an oral antihistamine, with no the need to seek emergency care or use adrenaline auto-injectable. Figure 4

As some patients were stung more than once, 35 accidental stings were observed during the first year of the maintenance phase. Figure 4

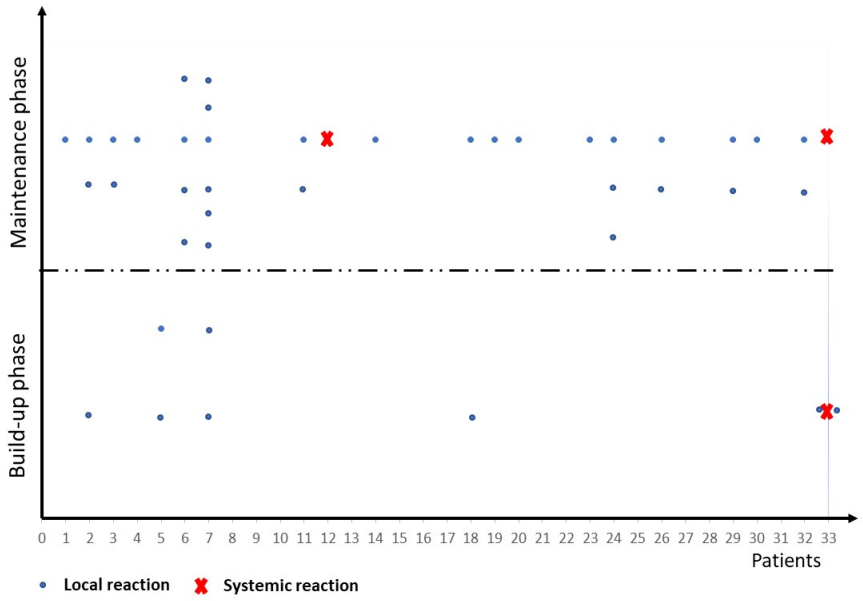

Figure 4: Distribution of accidental sting per patient during the build-up and maintenance phase according to the severity of the reaction.

The patient 7 draws attention, he lives in a rural area, and he was accidentally stung eight times, two times in the build-up phase, and six times in the maintenance phase, with only one local reaction in all events.

Considering only the patients who had a grade IV systemic reaction in the index field sting (n=9), one patient had two field stings during the build-up phase with local reaction. During the maintenance phase: three patients had field stings (one, four and two stings) with local reaction, and one patient had a single sting with a mild systemic reaction, whose hives improved after taking 20mg Bilastine.

Discussion

Fire ant stings are frequent in pediatric age: 32% to 54% of the surveyed population[25].

In this study, systemic reactions after fire ant stings were also more prevalent in patients (n=17) under 15 years (51.51%). If we expand the age group up to 20 years old, the frequency of the systemic reactions rises to 75.9%. Among Grade-IV reactions, the patient's age has not positively correlated with the grade of the sting reaction.

In literature, male sex is an independent risk factor for severe systemic reactions to the field sting. The effect of male sex in insect sting induced anaphylaxis presumably results from a selection effect. Because of a different degree of exposure, adult men are stung more frequently than women. They might, therefore, be at higher risk for sensitization or severe allergic reactions to honeybee or wasp venom. There is no data on fire ant venom[26]. In this study, there was no gender influence on the severity of the reaction; there was only a slight predominance of males: 51.5% over females: 48.5%, probably due to the greater exposure.

Concerning atopy, it is known that it is a risk factor for anaphylaxis triggered by food, exercise, and latex[27]. It has not yet been established that atopic disease increases the risk of anaphylaxis associated with *Hymenoptera* venom allergic reaction[28].

According to an international anaphylaxis consensus (ICON)[29]; cardiovascular disease and uncontrolled asthma are well-recognized risk factors for severe anaphylaxis in general. In the current study, there was no correlation between asthma with a Grade-IV systemic reaction.

Considering the previous systemic reaction, in literature, 18% of the mild systemic reactions in previous stings evolved into severe systemic reactions[30]. In the current study, the percentage was lower, 11.11%. In the same study mentioned above, a third of patients (24%) developed the same severity as the previous reactions[30]; meanwhile, we obtained a percentage of 22.22%, data similar to the literature.

Considering the previous large local reactions, Golden et al[31] observed that 7% of children who had large local reaction progressed to a systemic reaction in subsequent stings. Graft and collaborators[32] reported 2% of systemic reaction when they had large local reactions previously. Our findings showed that 11.11% of the patients had experienced a large local reaction in the previous sting. The vast majority (55.56%) reported only local reactions in previous reactions. Therefore, in our patients, there was no correlation between having a certain severity of reaction in previous stings and subsequently evolving to a more severe reaction in subsequent stings ($p = 1.207$). The vast majority reported only a local reaction in the sting prior to anaphylaxis.

In literature, a history of large local reactions carries a lower risk of a systemic reaction than in all other sensitized patients. When a large local reaction results after bee venom, the likelihood of anaphylaxis from a future sting is approximately 5%. For comparison, when there is a history of anaphylaxis from a previous Hymenoptera sting and the patient has positive skin test results to venom, at least 60% of adults and 20-32% of children will develop anaphylaxis with a future sting[33]. A fact that draws attention in our study is in 4 out of 18 patients, the first systemic reaction was already severe, and that these patients were not referred to a specialist for investigation. Only in the subsequent reaction, two patients were referred to a specialist for investigation, and the other two patients were not referred, looking for a specialist willingly.

Comparing the results of specific serum IgE measurements against fire ant venom with skin tests results in patients with grade-IV systemic reactions, our findings corroborate with the literature[34,35,36] that there is no correlation between the degree of sensitization and severity of the reaction.

Changes in specific IgE and IgG4 antibodies measurements

The levels of fire ant IgE antibody decreased in the period observed in 73.3% of the patients, while specific IgG4 increased in that period. Thus, the specific IgE/IgG4 ratio at baseline and 12 months after the maintenance phase showed significant differences.

After an initial increase in the first months of treatment, specific IgE levels tend to decrease during immunotherapy[37,38] and generally remain low even after discontinuation of treatment[39]. On the other hand, specific serum IgG4 levels increase during immunotherapy with venom[40,41,42]. It is known that high levels of specific IgG4 are positively correlated with the number of stings, previously described in beekeepers[43,44,45]. Thus, the specific IgG4 antibody is considered a tolerance indicator in allergic individuals because it has been suggested in the literature that these antibodies can block the interaction between the allergen and the specific IgE antibodies, thus preventing reactions with the participation of IgE. These effects should be reflected in the specific IgE/IgG4 ratio[46,47]. The explanation for this is due to the regulatory cell populations that form a suppressive environment: a slight decrease in the production of allergen-specific IgE and early switch to B cells to produce IgG4 and, consequently, an increase in IgG4 antibodies, which is a non-inflammatory agent in allergic disorders[7,48].

Serum tryptase levels

In literature, about 80% of patients with Muller's Grade-IV reaction to *Hymenoptera* venoms (bees and wasps) are diagnosed with mastocytosis. In these severe reactions, 20% of patients could not show clinical signs of mastocytosis[49]. Bonadonna et al[50] reported a correlation between a systemic reaction to *Hymenoptera* sting and mast cell tryptase. Three hundred seventy-nine patients presented a history of systemic reactions after being stung by these insects, 11.6% had serum mast cell tryptase that exceeded 11.4 ng/mL. In this group, the rate of anaphylaxis (Muller's grade IV) was 70.5%. Blum et al[51] confirmed these findings in a 5-year retrospective study, with 868 patients who had severe reactions after insect stings (758 patients had both: total IgE and baseline tryptase level above the reference values). Elevated basal tryptase (> 11.4 ng/mL)

was associated with severe systemic reactions (p <0.03). Due to this strong association, the guidelines on allergy to *Hymenoptera* venoms always recommend evaluating serum tryptase for these patients.

In this study, two patients (6.25%) presented a tryptase level higher than 11.4 ug/ml; however, due to the specific investigation of hematology in our hospital, the diagnosis of Systemic Mastocytosis was ruled out. As already mentioned, in literature, there is a high correlation between severe anaphylaxis and *Hymenoptera* stings but being reported by bees and wasps. There is no data on fire ant venom so far.

Adverse reactions during immunotherapy

Adverse events are frequently observed when unpurified extracts are utilized, and the aqueous formulations tend to cause more local reactions than depot diluents [52,53,54].

The main risk factors that provoke serious reactions in the literature[55] were very well-reviewed at each patient visit and controlled in this study: dosage error, administration of the injection without supervision by a trained professional, presence of uncontrolled asthma, the high degree of sensitivity, concomitant use of β-blockers, systemic reactions prior to immunotherapy and use of lots with new products.

When a local reaction was greater than 50mm, patients were instructed to use fexofenadine 180mg (adults), and for children, the dose was adjusted according to the weight presented. Only one adult patient took the oral antihistamine because she had a bother local reaction; other patients did not need the medication.

In a multicenter study[56] evaluating data from 840 patients, side effects of immunotherapy (systemic reactions) with venom (seventy-one percent were treated with Vespula-venom extract and 27% with honeybee-venom extract) were observed in a total of 20% of patients; 26,601 injections in 840 patients, systemic reactions were observed in 1.9% of the injections during the build-up phase and in 0.5% the maintenance phase (p <0.05). Most of these reactions were mild, and only a third of patients required medical treatment. A similar frequency of systemic adverse effects was observed in a published study analyzing data from 178 patients[57]. The build-up phase of venom immunotherapy has more adverse effects than the maintenance phase.

In our study, the systemic reaction rate during immunotherapy was lower than the literature, comparing extracts of honeybee or wasp venoms. Our results showed a systemic reaction in 6.06% during the build-up phase. It was also lower when compared by injection (0.017%). In the maintenance phase, there were no systemic reactions. The similarity remained in the slight severity of these reactions.

Thus, the severity of side effects due to immunotherapy does not necessarily correlate with the severity of the treated allergic disease symptoms. Systemic reactions induced by venom immunotherapy may occur, but most patients tolerate this treatment without relevant side effects.

Accidental Field stings

Reaction evaluation after a sting during or after the treatment (either by a challenge test with culprit insect or by accidental sting) is an available method to determine the degree of response to venom immunotherapy[58].

When the challenge test with culprit insect was performed in a series of patients undergoing immunotherapy with bee and wasp venom, about 75 to 85% [8,59,60] or even more 95% [61,62]of patients were protected.

Arseneau at el[20] evaluated 66 patients submitted to 1-day rush immunotherapy protocol using fire ant whole-body extract, and the conclusion was that the immunotherapy was efficacious (1 of 53 confirmed fire ant sting challenges (1.9%) resulted in a reaction) and had a low rate of systemic reactions.

In Brazil, for ethical reasons, we could not perform the provocation test with the culprit insect; it is possible only to observe reactions by accidental stings during treatment.

In this study, 20 patients were stung, 35 stings in total, as some of them were stung more than once.

During the build-up phase, the patient still has an increased chance of having systemic reactions if he is stung at that moment. The patient who had a systemic reaction in the build-up phase in our study had a mild reaction (only hives, with no need to seek immediate attention or use adrenaline auto-injectable).

Thus, we had one systemic reaction (in an accidental sting), out of 5 patients stung during this phase: 20% treatment failure.

During the maintenance phase, two patients were accidentally stung. They had systemic reactions (10.5%) and described as mild reactions: only hives, without the need to seek emergency care or the use of an adrenaline auto-injectable.

The percentage of reactions in the maintenance phase (10.5%) is lower than the percentage found in the literature, which considers approximately a 25% risk of a new generalized systemic reaction in subsequent stings in patients who received venom immunotherapy for 1 or 2 years[63].

The optimal length of treatment with fire ant immunotherapy is not known. The treatment with fire ant immunotherapy commonly used often is 3–5 years based on extrapolation from flying *Hymenoptera*immunotherapy data. A 3- to 5-year course of flying Hymenoptera (e.g., honey bee, hornet, wasp, and yellow jacket) venom immunotherapy has been shown to provide protection against systemic reactions[64].

Forester et al[65] grouped patients into those who received < 3 years (reduced course) and those who received > 3 years (complete course) of imported fire ant immunotherapy (IFA). All subjects on IFA immunotherapy received a maintenance dose of 0.5 mL of a 1:100 w/v concentration of IFA whole-body extract. No difference in the incidence or severity of systemic reactions to field stings after immunotherapy discontinuation for the two groups studied. The systemic reaction rate was low for both the complete and the reduced course groups (7 and 6%, respectively) after discontinuation of IFA immunotherapy.

Our patients will be in immunotherapy for 3 years. After this period, new data will be gathered.

Conclusion

Few studies have evaluated patients undergoing fire ant immunotherapy with whole-body extracts. Our study is unprecedented in the evaluation of clinical and laboratory data on these treatments.

The development of peripheral tolerance is the primary mechanism of immunotherapy. In this study, we verified a significant difference at the baseline and after one year of the maintenance phase in a specific IgE/IgG4 ratio, reinforcing the hypothesis that the IgG4 antibody has a mode of action "blocking" the specific IgE. It is important to note that allergen-specific IgG can interfere not only in elucidating allergic responses triggered by IgE in effectors but also in presenting antigen IgE-mediated to T cells. It may be essential to decrease the responses of allergen-specific TH2 cells. It is essential to consider not only the individual values of each antibody but the ratio between them at baseline and during immunotherapy. In this aspect, it would be interesting to obtain further studies on specific T cells' behavior during immunotherapy with fire ant venom.

The maintenance dose of 0.5mL in the concentration 1: 100 wt/vol (Hollister Stier, USA) of a mixture of whole-body extract of*Solenopsis invicta* and *richteri* was effective because of the 20 patients accidentally stung during the treatment (1 year of maintenance dose), two patients (10.5%) had a systemic reaction (urticaria), with improvement after taking an oral antihistamine. Adverse reactions to immunotherapy were rare.

The lessons learned in this study should help to improve both the effectiveness and the maintenance of tolerance to fire ant immunotherapy. This treatment proved to be safe and effective.

Patients will complete treatment for three years from the maintenance dose, and patients will be followed up after discontinuation of treatment.

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