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Evaluation and validation of a bee venom sting challenge performed by a micro-syringe

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ARTICLE INFO

Article history:

Received for publication July 13, 2012.

Received in revised form September 5, 2012.

Accepted for publication September 5, 2012.

ABSTRACT

Background: The honeybee sting challenge is considered a reliable procedure to evaluate the efficacy of specific immunotherapy, but it is difficult and impractical to perform in clinical practice, because live insects are required.

Objective: To assess the feasibility and reliability of a challenge test using a micro-syringe, and compared the procedure with sting challenge.

Methods: Patients on bee venom immunotherapy and without systemic reactions at field sting were enrolled. They underwent a sting challenge with live bee, and large local reactions were assessed up to 48 hours. Those patients displaying systemic reactions at the sting challenge were excluded from the syringe challenge for ethical reasons. The syringe challenge was done by injecting 0.5 μ L fresh unfiltered bee venom at 2 mm depth (the length of the sting left by a bee). The same follow-up as at the first challenge was performed. Bee-specific immunoglobulin E (IgE) and tryptase were measured after each challenge.

Results: Nineteen patients underwent the sting challenge with live bees. Four had immediate systemic reactions (urticaria or asthma) and were excluded from the second challenge. The remaining 15 patients with large local reaction underwent the syringe challenge. No significant difference was seen in the maximum area of the large local reactions between the challenge with live bees and the syringe challenge. Also, no change was seen in tryptase and specific antibodies.

Conclusion: This preliminary study suggests that the micro-syringe challenge with honeybee venom is feasible and produces results indistinguishable from those of the traditional sting challenge.

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Introduction

The diagnostic procedures for hymenoptera venom allergy (HVA) are quite uniform and universally accepted. They involve clinical history, skin prick tests, intradermal tests, and measurement of serum-specific IgE.^{1,2} A detailed etiological diagnosis is obviously mandatory for the prescription of the appropriate venom immunotherapy (VIT), which is highly effective in protecting from severe reactions at subsequent field re-stings.³ Among the second-line diagnostic tools available, the sting challenge with live hymenoptera remains a matter of discussion.^{4,5} The sting challenge allows reproduction under controlled conditions of the effects of a field sting, but it is not easy to perform, it

is time consuming, and it may result in severe systemic reactions.⁶ In addition, the sting challenge has been reported to be not fully reproducible or predictive for subsequent reactions.⁷ Thus, sting challenge is not considered a feasible diagnostic approach for HVA.⁸ Conversely, the sting challenge maintains a certain value in research settings, or even to confirm the protection achieved by VIT in subjects who are at risk for field re-stings, or to evaluate whether the maintenance dose is sufficient to achieve protection.^{3,8} Sting challenge is mainly used to study honeybee venom allergy, because bees inject all of the content of their venom sacs.^{9,10} Thus, the amount of venom injected can be assessed quite accurately, and the challenge can be objectively documented by the presence of the sting in the skin. This is not true for vespids, which inject variable amounts of venom, making the sting challenge less reproducible. Thus a simplified method not requiring the live insect would be advantageous and desirable.

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Disclosures: Authors have nothing to disclose.

The aim of this study was to assess the performance of an artificial sting challenge with honeybee venom, performed with a micro-syringe in subjects with local sting challenge reactions. The results of the syringe challenge were compared with those of the challenge with live bees.

Methods

The study was conducted in patients (mostly beekeepers) with HVA currently receiving VIT for honeybee, and without systemic reactions after field stings during VIT. As a first step, patients underwent a standard sting challenge with a live bee. They were kept under strict medical observation to identify and manage local/systemic reactions. Those patients who did not develop systemic reactions subsequently underwent the micro-syringe challenge. They were again observed for at least 1 hour, and reactions were carefully recorded. The study was approved by the Rimini Hospital Ethical Committee, and all patients signed an informed consent before the study.

Patients

Subjects were enrolled who were previously diagnosed bee venom allergy only, on maintenance with bee VIT for at least 1 year, and without systemic reactions after field re-stings. The diagnosis of HVA had been made according to current guidelines, by percutaneous/skin prick test and specific IgE assay.¹ Also, VIT was prescribed according to current recommendations. VIT for bee venom involved a rush buildup in 4 days with a cumulative dose per day of 4, 19, 65, and 100 μg , followed by a maintenance with 100 μg venom given every 4 to 6 weeks.¹¹ The same commercial venom product (Anallergo SpA, Florence, Italy) was used in all patients. All subjects appeared to be efficiently protected as evidenced by the absence of systemic reactions at subsequent field stings. The challenges were performed in all patients no sooner than 1 month from the last field sting. Two volunteers without HVA, with negative diagnostic tests, and without large local reaction or systemic reactions at previous field stings, underwent the same procedures as controls.

Sting challenges and measurements

The standard sting challenge with live bee was performed according to Golden et al.¹⁰ Patients had an intravenous line placed and were kept under continuous medical observation for 1 hour after challenge. The course of LLR, if any, was monitored at different intervals (30 minutes, 1 hour, 24 hours, 48 hours). No medication was allowed during the 48-hour observation period, unless a systemic reaction occurred. In such a case the appropriate therapy was immediately given.

The microsyringe challenge was performed only in those patients who did not develop a systemic reaction after the challenge with the live bee. At least 1 month elapsed between the first and the second challenge. For the micro-syringe challenge, a Hamilton 2.5- μL syringe was filled with 0.5 μL bee venom (Fig 1). Bees had been preserved at -80°C for 2 to 4 months. At the time of challenge, they were defrosted, the stinging apparatus was dissected, the venom sac gently squeezed, and the venom collected from the sting by means of a capillary tube. The volume of venom to be injected was chosen by calculating the mean of the amount of venom extracted from 200 bees. Thus, both the amount and the composition of the venom strictly reproduced the naturally occurring sting. The needle was inserted perpendicularly into the skin of the volar surface of the forearm for 2 mm (using a calibrated spacer), and the venom was injected in 5 to 10 seconds. The depth of 2 mm was established by measuring the length of the sting that remained in the skin after the naturally occurring stings.

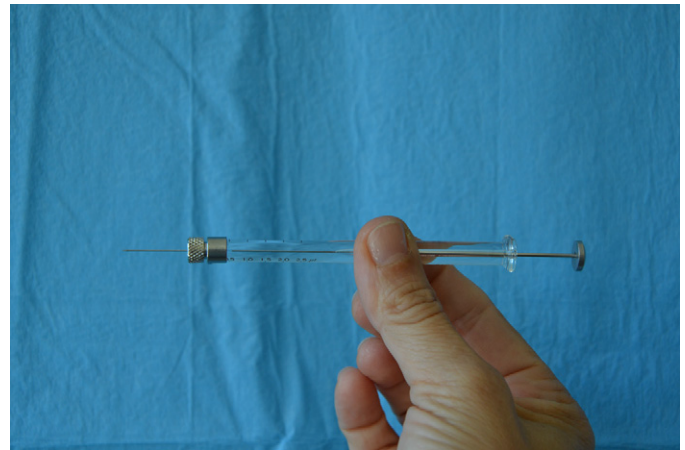


Figure 1. The micro-syringe used for the challenge.

LLRs were evaluated by measuring the maximum area in square centimeters reached by the wheal during the observation period. The patients were also required to score on a visual analog scale from 0 (absent) to 10 (intolerable) the intensity of the pain perceived at challenges. Serum tryptase and bee-specific immunoglobulin E (IgE) were assayed after each challenge by commercial immunoenzymatic methods (Thermo-Fisher, Upssala, Sweden).

An electrophoretic analysis (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was also performed to assess the composition of the venom extracted from the frozen bees in comparison with the commercial extract used for diagnosis and specific immunotherapy.

The differences between the results of the 2 challenges were evaluated by means of an unpaired Student *t* test, considering $P < .05$ as significant.

Results

Nineteen patients (14 male, mean age 45 years) with single sensitization to bee venom, currently receiving honeybee VIT (duration of maintenance 12–138 months), and with no systemic reactions at field sting, were studied. The demographic and clinical data of the population are summarized in Table 1. Of 19 patients, 4 had immediate systemic reactions at the sting challenge with the live bee: 2 grade I (urticaria) and 2 grade III (asthma). They were treated with an appropriate therapy, with the prompt remission of symptoms. Those 4 patients were excluded from the subsequent micro-syringe challenge. Fifteen patients (13 with LLRs and 2 negative) underwent the micro-syringe challenge. In the 15 subjects eligible for the second challenge, the maximum area of

Table 1
Demographic and clinical data of the studied population

Sex (M/F)	14/5
Mean age, years (SD)	45 (± 14)
Age range, years	28–67
Intradermal test positivity	
1 $\mu\text{g}/\text{mL}$	5
0.1 $\mu\text{g}/\text{mL}$	6
0.01 $\mu\text{g}/\text{mL}$	3
0.001 $\mu\text{g}/\text{mL}$	5
Mean honeybee-specific IgE (range)	4.4 kU/L (0.1–12.4)
Mean serum tryptase (range)	5.7 $\mu\text{g}/\text{L}$ (2.1–12.7)
Reaction before VIT	
I	8
II	1
III	6
IV	4
Mean VIT duration (range)	48.1 months (12–138)

Abbreviations: IgE, immunoglobulin E; VIT, venom immunotherapy.

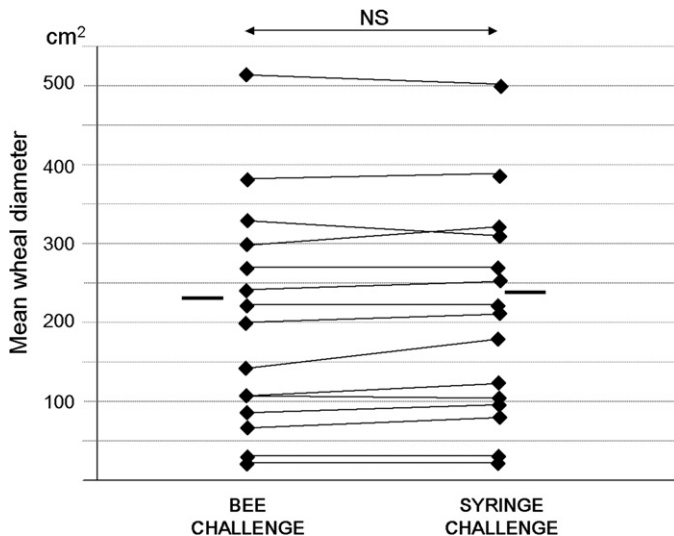


Figure 2. Maximum area of the LLR after the challenge with live bee and syringe challenge (medians are indicated as thick horizontal lines).

the LLR did not significantly differ between the bee sting challenge and the micro-syringe challenge ($231 \pm 125 \text{ cm}^2$ vs $240 \pm 119 \text{ cm}^2$; $P = .09$), as shown in Figure 2. The 2 subjects with negative challenge with the live insect were negative with the syringe challenge. No significant difference was seen in the intensity of the perceived pain at the challenge between bee sting and micro-syringe (3.4 ± 0.7 vs 3.1 ± 0.9 ; $P = .4$). No significant difference could be found in serum tryptase and specific IgE as measured at baseline (before starting VIT) and after the 2 challenges, as shown in Figure 3. An overall decrease in honeybee-specific IgE was observed during VIT, but this decrease did not reach statistical significance. The 2 control patients displayed negative results at both challenges. Finally, there was no appreciable difference in the protein profile between the venom used for the syringe challenge and the commercial one, used for diagnosis and immunotherapy (Fig 4)

Discussion

The sting challenge procedure with live insects is still a matter of debate, mainly because of its nonoptimal reliability and predictive value.^{8,12–14} Conversely, sting challenge remains the only procedure to experimentally assess the efficacy of VIT and to confirm that a protective maintenance dose is administered. In addition, the

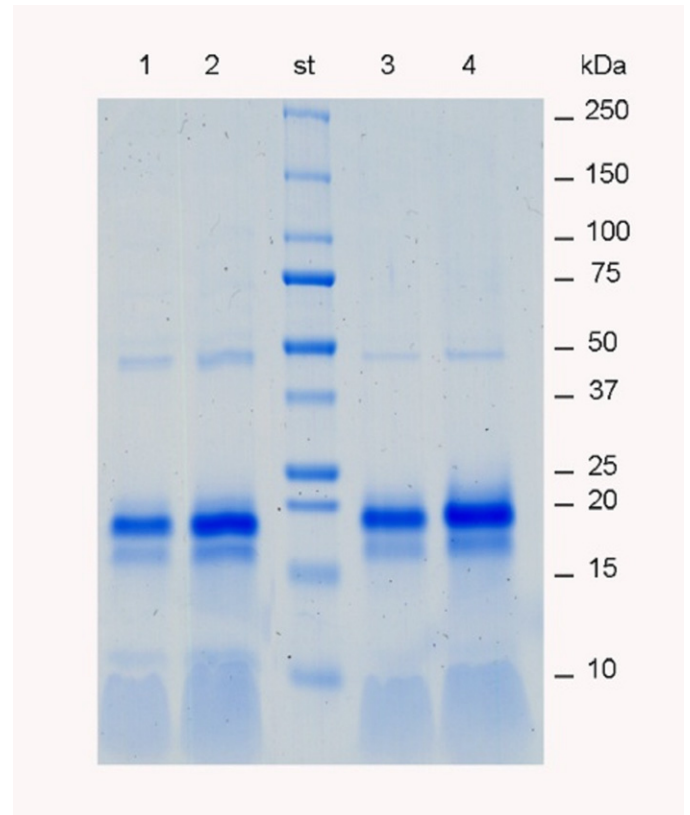


Figure 4. Gel electrophoresis of the venom extracted from frozen bees (lanes 1 and 2), the commercial extract (lanes 3 and 4), St: molecular weight standard.

sting challenge maintains a central role in experimental studies, whereas the effects of VIT need to be measured under controlled conditions, and without waiting for a field re-sting.^{15,16} Under these selected conditions, the main limitations of the sting challenge with live insect are the need for specialized personnel to manage the insects, the time consumption, and possibly the costs. Thus, an “artificial” challenge procedure would solve some of those problems and would allow a wider use of the diagnostic test. Indeed, attempts have been made to replace the sting challenge with live insects,¹⁷ but the performance of such approaches was not studied in depth.

We set up an artificial challenge procedure, using a micro-syringe device, to best mimic a natural bee sting. The amount of venom and the depth of the stinger were carefully established by measuring the quantity of venom contained in the venom sacs, and by measuring the length of the sting left in the skin after a naturally occurring sting. We then compared the reaction obtained with a stinger challenge with that obtained with the micro-syringe. For ethical reasons, only those patients allergic to bee venom, who appeared efficiently protected (only LLR at field re-sting) by specific VIT, were studied. The results of the 2 types of challenge were indistinguishable in term of LLR area, and no change was seen in the relevant immunological parameters. However, no assumption could be made concerning the predictive value of the test, because the micro-syringe challenge was not performed for ethical reasons in those patients who had systemic reactions at the bee challenge. The micro-syringe challenge was overall well tolerated, and no systemic reaction was observed. Bee venom was chosen to validate this first approach, because the amount of venom injected is quite reproducible, but the procedure could be proposed, at least in principle, also for vespids. Another practical advantage of this technique is that the venom can be easily frozen or lyophilized for

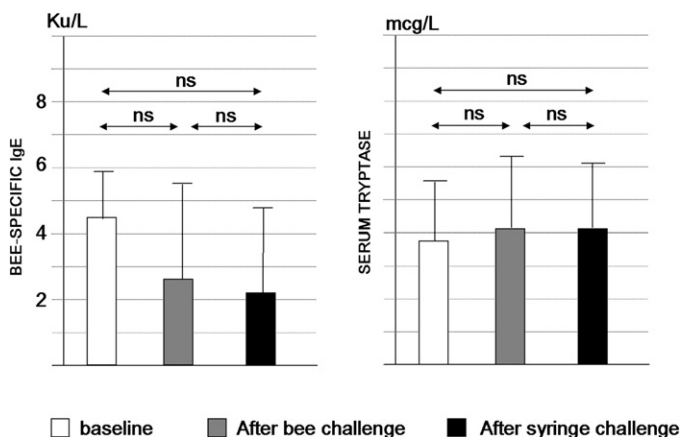


Figure 3. Serum bee-specific IgE (left) and serum tryptase (right) before VIT, within 1 hour after sting challenge, and after micro-syringe challenge (means and standard deviations are reported).

use after preparation. This would allow, in the future, a more routine use of the challenge, which remains a procedure to be performed by well-trained personnel.

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