

The Lights and the Shadows of Controlled Sting Challenge with Hymenoptera

Short Title: Sting Challenge with hymenoptera

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Abstract

Hymenoptera venom immunotherapy (VIT) is a treatment which has been shown to be effective in the protection of individuals with systemic allergic reactions caused by Hymenoptera stings. The need for a tool which shows the degree of protection afforded by VIT and the lack of useful biomarkers, has converted the Sting Challenge Test (SCT) into the *gold standard* for this disorder although its use has both lights and shadows. SCT with Hymenoptera is a test consisting of causing a real sting in a patient diagnosed with an allergy to the venom of the stinging insect and who normally is undergoing treatment with specific immunotherapy. In Spain, SCT is included in the list of services offered by some hospitals and forms part of their habitual clinical practice. This review aims to analyze the strengths and weaknesses of this test and describe the standardized procedure and necessary resources, based on the experience of a group of Spanish experts and a review of the literature.

Key words: Sting challenge test. Hymenoptera venom allergy. Venom immunotherapy.

Resumen

La inmunoterapia con veneno de himenóptero (ITV) es un tratamiento que se ha mostrado eficaz en la protección de sujetos con reacciones alérgicas sistémicas por picaduras de himenópteros. La necesidad de una herramienta que demuestre el grado de protección proporcionada por la ITV, y la ausencia de biomarcadores útiles, convierte a la Prueba de Provocación con Repicadura (PPR) en el *gold standard* en esta patología, con sus luces y sus sombras. La PPR con himenópteros es una prueba que consiste en provocar una picadura real, a un paciente que ha sido diagnosticado de alergia al veneno del insecto picador y habitualmente está en tratamiento con inmunoterapia específica.

En España, la PPR se incluye en la cartera de servicios de algunos hospitales, formando parte de su práctica clínica habitual. Esta revisión trata de analizar las fortalezas y debilidades de esta prueba, integrando el procedimiento estandarizado y recursos necesarios, basándose en la experiencia de un grupo de expertos españoles y en la revisión de la literatura.

Palabras clave: Prueba de provocación con repicadura. Alergia a veneno de Himenóptero. Inmunoterapia con veneno.

Introduction

IgE-mediated allergy to Hymenoptera venom manifests as a generalized allergic reaction of variable severity which can be potentially life-threatening and which occurs following an insect sting [1]. Hymenoptera venom immunotherapy (VIT) is a treatment which has been shown to be effective in protecting allergic individuals from stings in 77-85% of cases of bee venom and in 91-96% of cases of vespid venom [2], reducing both morbidity and mortality and improving patient's quality of life [3]. Although during VIT patients tolerate doses of venom greater than that contained in the venom sac of the insect, there is no guarantee that individually they will tolerate a live insect sting [4]. This, together with the uncertainty that the absence of spontaneous stings generates in some patients, highlights the need for a tool to demonstrate the degree of protection provided by VIT.

Sting Challenge Test (SCT) with Hymenoptera consists of causing a real sting in a patient who has been diagnosed with an allergy to the venom [4,5] and currently is only recommended in patients undergoing VIT.

SCT has been used to assess the efficacy of immunotherapy since its inception in the 50s by Mary Hewitt Loveless and William R Fackler [6]. In 1978 it was used in the comparison of VIT with purified venom against body extract [7]. Over the following 40 years, it has been used in studies which monitor the time course of protection in patients treated with VIT, including follow-up after discontinuation of the treatment [8,9] as the reference technique for the phenotyping of patients allergic to bee venom [10]. Studies of patients not treated with VIT but subjected to SCT have also been published; mention must be made of a series of 138 patients in whom the risk factors involved in the appearance of reactions following stings were analyzed [11]. Its value as a diagnostic tool

has also been studied [12] although the ethical debate surrounding this practice is a limiting factor [1,13] as is explained below and for this reason currently SCT is not indicated as a diagnostic method.

In Spain, the diagnosis and etiologic and preventive treatment of allergic reactions due to insect Hymenoptera stings are a competence of the specialty of allergology [14,15]. SCT is included in the list of services offered by some hospitals, especially in those which have more experience in the management of allergic reactions to stings, and thus forms part of their habitual clinical practice [16].

The aim of this paper is to review the strengths and weaknesses of SCT in Hymenoptera venom allergy as well as to establish the standardization of the technique from the point of view of the safety, availability and applicability to habitual clinical practice based on the experience of a group of Spanish experts and a review of the literature.

Indications and contraindications for the SCT

Currently the test is used in patients under treatment with VIT who have reached the maintenance phase and several years after VIT to monitor its efficacy [17]. Given the lack of in vitro biomarkers capable of predicting the effectiveness of VIT, at present SCT is considered the gold standard for evaluating the risk of systemic reactions during and after specific treatment with immunotherapy [1]. The remaining indications and the contraindications for the SCT are listed in table 1.

Procedure and necessary resources

Obtaining and handling the insects

The Hymenoptera responsible for the immense majority of stings in Spain are the honey bee (*Apis mellifera*); the and wasps *Polistes dominula*, *Vespula germanica* [18]; and *Vespula vulgari*; and rarely other species such as *Vespa crabro*, *Dolichovespula* spp, *Bombus* spp. (bumblebee). The recent establishment of other species such as *Vespa velutina* in the north of Spain pose, a diagnostic and therapeutic challenge to which allergologists must give an answer [19].

The SCT is performed with the species of insect responsible for the clinical reaction and should be conducted at the time of year when the insect responsible for the sting is available. Usually, the most used frequently species for SCT are *Apis mellifera* and vespids (*Vespula germanica*, *Polistes dominula*). It is essential to obtain the appropriate entomological identification.

Currently in Spain there is a company [20] that is able to supply exemplars of *Apis mellifera* and vespids (*Vespula germanica*, *Polistes dominula*) which ensures the correct identification of genus and species. This company is directed by a biologist specialized in entomology who captures, identifies, and supplies the Hymenoptera by sending them to any place in Spain. Currently, it does not make international shipments.

The bees should be obtained from hives and not the open countryside. Specimens used for SCT should be workers more than 2 days old. Beekeepers may be involved to obtain live honeybees, or they could be supplied by the company mentioned above. The bees must be kept in an appropriate plastic container with a small opening through which they

can breathe and with feeding material (water and honey from the same hive or a sucrose concentrate) which will allow them to survive for a few days if the test is not to be performed the same day.

The identification of wasps is a much more complex matter, particularly in those regions where more than one species of allergenic vespids coexists. It should be identified by an expert (entomologist, biologist or trained allergologist). No notable differences have been observed between SCT with insects obtained at end of spring or in the middle of autumn [12].

It is important to have a sufficient number of specimens of vespids in an appropriate plastic container with a small hole so they can breathe. A part of the nest as a food source to increase survival should be included (this is possible only with aerial nests like those of *Polistes dominula* because the extraction of subterranean nests it is not realistic). The container must be kept in a cool and dark room. This will allow the insects to survive for up to 4 weeks. The specimens used in the test should be progressively warmed up and allowed to become active [21].

The insects must be handled in a well-ventilated room and made lethargic by the application of CO₂ (used in the hospital for laparoscopies) applied through the ventilation holes in the containers for a few seconds until the insects remain immobile to cut off their wings and hind legs so as to avoid accidents inside the hospital. This same effect can be obtained by exposing the insects to cold. The insects are then placed in individual ventilated plastic tubes until their use in the SCT.

Clinical Setting and Preparation of the patient

SCT requires an appropriate healthcare setting and controlled conditions. Although the technique is safe, the need to control risks requires SCT to be performed in hospitals which have available trained staff both in the technique and in the management of severe allergic reactions [22,23].

The room for the test, the material (Photograph 1) and the necessary staff are listed in table 2.

Once patients have been selected bearing in mind the indications and contraindications for this test they should be informed in detail of the possible risks and consequences and should sign the corresponding specific informed consent form.

Patients should undergo an initial assessment and examination and it is necessary to suspend during an appropriate length of time those drugs which might inhibit the reaction such as corticoids, antihistamines and anti-IgE antibodies. Regarding treatment with angiotensin-converting enzyme inhibitors and β -blockers, recent studies have shown that these drugs do not increase the severity of reactions following spontaneous or controlled stings [24].

We recommend that patients do not smoke or drink alcohol or fats for 6 hours prior to the test and for one hour afterwards.

Chronic diseases should be controlled. It should be checked that patients have not been stung recently and are adequately tolerating the VIT.

Heart rate, blood pressure, oxygen saturation should be monitored and in asthmatics, PEF and FEV₁, which should be measured before the test, and in all cases a peripheral intravenous catheter should be placed on the opposite arm to that being used for the SCT.

After the peripheral line has been set up, a sample of peripheral blood should be taken for the determination of baseline levels of mediators such as tryptase.

The SCT

When the insect has recovered its baseline level of activity, it should be held by the thorax with tweezers and placed on the forearm (volar face) of the patient with slight pressure on the abdomen against the skin so as to force the sting.

In the case of bees, the stinger will remain embedded in the skin of the patients due to the lateral barbs which give it the shape of a harpoon and the stinger attached to the venom sac which will continue to inject venom. The insect should be left in place for 30 seconds and then the body and the venom sac of the bee should be removed. Bees only sting once and die from eventration as the stinger is left embedded (Photograph 2A).

In the case of wasps (Photograph 2B), when the patient notices the sting, the insect should be forcibly held against the forearm for 30 continuous seconds so that sufficient venom is injected because usually the wasp does not leave the stinger embedded although in some cases *Vespula* spp. it does [25]. Thus, the wasps may normally make several stings in the forearm moving the abdomen.

The clinical status of the patient should be monitored for 2 hours, at 15-30-60-120 minutes after the sting. Should symptoms appear, vital signs should also be taken at that moment.

The outcome of the SCT:

The development of an erythematous reaction after a few minutes in the area of the sting is the only objective indicator of the actual inoculation of the venom and if this reaction does not occur, we should interpret this as the venom has not been injected and the tests should not be considered valid [22]. The papule can be measured quantitatively after 15 minutes. The appearance of a local or extensive reaction is usual after a re-sting. A positive response will consist of the appearance of systemic symptoms typical of an IgE-mediated skin and/or anaphylactic reaction, in general reproducing those experienced by the patient in previous reactions. The symptoms should be graded following the classification for systemic reactions, serum tryptase levels determined during the acute episode, and the patient should be reassessed to determine the need for changes in the protocol, VIT dose and duration of the same.

A negative response would be the absence of systemic symptoms.

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SCT as a possible biomarker

Among the tools available for the follow-up of VIT, there has been a search for useful biomarkers for monitoring its effectiveness.

Specific IgE (sIgE):

sIgE against the venom undergoes an initial rise and a subsequent decrease throughout the maintenance of VIT, remaining at low levels for several years following interruption of the treatment [26,27]. A decrease in levels of sIgE against individual allergens in relation to VIT has also been observed [28]. In spite of this decrease, these values rarely

become negative [26]. The coexistence of detectable levels of sIgE and tolerance of stings in some patients receiving treatment and the lack of differences in changes in sIgE between patients successfully and unsuccessfully treated, render this biomarker inappropriate for monitoring the risk of patients undergoing VIT for future stings [1,3,29].

Specific IgG4 (sIgG4):

sIgG4 tend to rise during VIT to a variable extent from one patient to another [30]. Some studies have linked the rise in sIgG4 with the effectiveness of VIT [31]. A decrease in sIgG4 levels following the interruption of VIT has been found although such a decrease has been found to have no effect on the protection obtained against stings [31]. In any case, there is no evidence as to a correlation between results of the SCT and levels of sIgE, sIgG4 or the ratio between sIgE and sIgG4 [32].

The basophil activation test (BAT):

BAT has shown a good correlation with results from spontaneous stings [33] and SCT [34], although due to the lack of standardization and its methodological complexity, its use is currently restricted to research [35]. In one study Hausmann et al. found a sensitivity and a negative predictive value of 100% but the specificity and positive predictive values were 87% and 50%, respectively, when the BAT was compared with SCT with live insects, which led the authors to conclude that the BAT could substitute SCT in cases in which formal contraindications exist [36]. A parallel situation has been observed between the expression of CD63 by basophils and protection of VIT in children allergic to bee venom and this may predict reactions to bee venom in new stings once immunotherapy is finished [34,37].

Although sIgE, sIgG4, and BAT have been shown to be useful in different aspects related to the tolerance of stings by patients treated with VIT, none of these techniques provides the same information as SCT, which is why SCT is considered the *gold standard* for monitoring the effectiveness of VIT and is thus, at present, an irreplaceable tool [1].

SCT as a programmable procedure

Some authors have based the effectiveness of VIT on the response to spontaneous stings but this is something that we do not know when it will occur and indeed, it could even never occur. Furthermore, a spontaneous sting may not guarantee a sufficient amount of venom, given that the patient rapidly frightens away the insect on being stung in the case of bees and with the sting lasting only 1-2 seconds in the case of wasps.

One of the advantages of the SCT is that it can be programmed for those patients undergoing VIT at different time points after the maintenance dose has been reached in order to assess its efficacy. Tests have been performed in such initial phases as a week after reaching the maintenance dose [38] to several years after its first use [26,39] and in general before finishing VIT [40]. In these cases, a positive SCT result allows the different factors associated with the failure of the VIT with bee venom to be identified [41] and strategies to be modified. SCT can also be programmed one or several years after the discontinuation of immunotherapy with the aim of verifying the patient's protection [40].

SCT would allow adjustment of the dose of VIT

A positive SCT in patients undergoing VIT allows the identification of those who need a larger dose than the conventional 100 µg and therefore the increase of the dose to 200

µg or more for protection to be reached, as has been reported [42]. In such cases, SCT allows the effectiveness of the dose to be verified.

SCT as a safe procedure in patients undergoing VIT

Much has been written about the possibilities of SCT causing serious anaphylactic reactions. This issue will be dealt with in the shadows section. However, here we would like to mention that as far as we know, no fatal reaction caused by SCT has been reported in patients treated in a hospital setting. Furthermore, it has been documented that the majority of systemic reactions caused by SCT have been shown to be less severe than the index sting [21,43]. Controlled SCT in a hospital setting will always be safer than a spontaneous sting in an uncontrolled place and the option of not performing the test in patients under clinical conditions that do not allow it is always possible.

The SEAIC has issued documentation on diagnostic and therapeutic procedures (RESCAL) classifying them into three levels of risk (A-B-C). SCT performed in uncomplicated cases of patients treated with immunotherapy has been classified as being at risk level B (more complicated procedures, procedures lasting more than two hours involving a moderate to high risk). SCT is assigned to level C in patients with comorbidities (highly complex tests requiring constant supervision and monitoring by specialist nursing and medical staff and/or extreme risk for the potential severity of the reaction or the existence of comorbidities) [16].

SCT is useful in patients with mastocytosis

In the case of patients allergic to Hymenoptera venom with mastocytosis, VIT is recommended indefinitely [44] and the need to know its efficacy by SCT is even more important than in the rest of patients given the severity of reactions in this group; in such

cases, the technique should be performed with the same safety measures as in the rest of patients [41,45].

Although mastocytosis has been identified as a risk factor for the failure of VIT, studies in which SCT has been performed conclude that systemic reactions, when they occur, are less severe and that VIT confers greater protection if the maintenance dose is increased [44,46,47].

SCT provides the possibility of in vivo research

SCT also opens up the possibility of studying in vivo the physiopathology of anaphylaxis by the monitoring of objective clinical, immunologic and biochemical events which occur in a programmed generalized allergic reaction [48,49]. Using this technique, the generation of FXIIa-C1inh, kallikrein-C1inh complexes as well as the degradation of high molecular weight kininogen [50]; or the levels of different mediators such as histamine and tryptase, among others, and endogenous vasopressors [11,51] have been reported. These would compensate for the vasodilation and extravasation of fluids which occur in anaphylaxis and would explain the ability of some patients to spontaneously recover after experiencing it without receiving any treatment [48]. Although it is not the most widely accepted recommendation, the SCT has been used to study the natural history of allergy to venoms. In one study, Golden et al. analyzed the frequency and severity of reactions to stings of different species of *Vespula* in 111 adults, most of whom had experienced slight to moderate systemic reactions due to *Vespula* stings and were therefore sensitized to this venom. A total of 175 serial SCT were performed with 72% being negative and the rest less severe than the previous spontaneous stings [12]. In another study [52], 92 children allergic to Hymenoptera venom were subjected to serial SCT after which

vaccination was recommended in 13, thus confirming, thanks to the use of SCT, the trend towards spontaneous resolution of the disorder in a large part of the pediatric population. The controlled exposure to Hymenoptera venoms has also been used in laboratory animals to determine the role of the adaptive type 2 immune response. The findings support the hypothesis that IgE and mastocytes help to protect the host against the toxic effects of the venom [53].

SCT improves quality of life

Another point that supports the use of SCT is its influence on the quality of life of those patients who have experienced systemic reactions to Hymenoptera stings and who live and work in settings where insects are present. Anaphylactic reactions following a bee or wasp sting may lead to a considerable decrease in the quality of life of those people who suffer them. These reactions have a substantial impact on daily activities in the open air (including professional activities) and may lead to symptoms of anxiety or depression [54]. To evaluate this reduction in quality of life in people who have experienced systemic reactions after Hymenoptera stings, questionnaires that evaluate general aspects of health (HRQOL) or specific issues (VQLQ) have been used [55]. Recently it has been demonstrated through the use of validated questionnaires that those vaccinated patients who tolerated SCT had a better quality of life than patients receiving VIT but who had not undergone this test [56,57].

The shadows of controlled sting challenge with hymenoptera

SCT as diagnostic tool

Sometimes the usual diagnostic tools are not enough to ensure the diagnosis, so the usefulness of SCT as a diagnostic tool is a matter of debate [1].

As is well known, a negative isolated SCT does not predict that subsequent tests will also be negative [23,29,58]. In the case of untreated allergic patients, to the risk of a serious reaction produced by SCT would have to be added the uncertainty of an isolated negative result. Therefore, the decision to restrict immunotherapy in a patient with an isolated negative SCT could have important consequences.

With the aim of evaluating the diagnostic value of SCT and recommend treatment with immunotherapy in patients allergic to Hymenoptera venom with systemic reactions, several authors have performed tests sequentially on the same patients to assess the reproducibility and negative predictive value of SCT, comparing the results with a second SCT or stings in the field. Golden et al. demonstrated that up to 11 of 37 patients with an allergy to *Vespula* venom with a negative result in a first SCT and without VIT presented systemic reactions following a second SCT [12]. In a study by Franken et al. of the 61 untreated patients allergic to *Vespula* venom, given a first SCT with negative results and of these, 21% had a positive result on a second SCT [59]. Blaauw et al. performed a SCT on 136 patients allergic to bee venom, of whom 76 had a negative result; of these, 41 patients subsequently received a sting in the field and 6 of them (14,6%) had a reaction. The authors also performed a SCT on 343 patients allergic to wasp venom of whom 284 patients were negative; of these, 127 patients were stung in the field and 13 patients (10,2%) had a reaction. Based on these results, the authors report that the negative predictive value of a SCT, with respect to subsequent stings in the field, is 85.4% in the case of bees and 89.9% for wasps [60].

Risk of the SCT in patients without VIT

When faced with the decision of indicating a SCT for a patient allergic to Hymenoptera venom, it is crucial to bear in mind the aspects related to safety, including the possibility of boosting sensitization.

In large series including patients allergic to Hymenoptera venom, it has been demonstrated that the reaction presented at the SCT in untreated patients is less severe than that reported by the patients in the clinical history and that the severity of the expected reaction is greater in those patients with a history of previous severe reactions [12,60]. In this regard, Blaauw et al. found that the prevalence of severe systemic reactions (grade III and IV in Müller's classification) following SCT was 24% for *Apis mellifera* and 8% for *Vespula*. In those patients with a history of severe reactions, the SCT presented the same severity as the previous reaction in 25% of patients allergic to *Apis mellifera* and in 15% of those allergic to *Vespula* [60]. In the study by Franken et al. 46% of patients who reacted with a second controlled SCT but not with the first, experienced anaphylactic shock which required the use of vasoactive drugs [59].

Given the data described in the last two sections, SCT is not habitually used as a diagnostic tool in patients not receiving VIT.

SCT could cause aversion

Not all patients with a history of allergy to Hymenoptera venom accept having a SCT with a live insect. One study reports that 26% of patients refused [8], although no in-depth study has been performed into the reasons for this. In any case, we believe that the key factor is the amount of information given to the patient and the confidence that patients receive in their healthcare setting.

Use of a live insect

Another problem with SCT is the need to use live insects. SCT is essentially different to the rest of allergology challenges because the live insect provides certain irreplaceable biological properties and because it presumably inoculates the entire content of its venom sac in one motion. It is considered that least 90% of the venom sac contents are delivered within 20 seconds [61].

Attempts have been made to reproduce the sting using subcutaneous and intracutaneous injections but with poor results [21]. Better results have been obtained using injections with a micro-syringe (0.5 μ L of pure venom) at a depth of 2 mm which could correspond to the conditions of a spontaneous sting, although the technique is arduous [13].

The reason why the venom of the live insect reproduces the reactions better than purified venom is unknown, although it has been postulated that the presence of low molecular weight substances such as vasoactive amines may influence the development of the allergic reaction [62].

Two important aspects which influence SCT are:

- a) Obtaining the insect and identifying it correctly. This has already been mentioned previously.
- b) Lack of quantification of the venom in SCT.

Unlike other controlled challenge techniques, SCT cannot be performed in a progressive fashion quantifying the amount of venom administered by the insect [22] and it is impossible to conduct blinded studies.

The different characteristics between insects, such as bees and wasps, may affect the result of SCT [1]. With each sting, bees inject a volume of venom of between 50 and 140 μg , a quantity much larger than vespids (1.7–3.1 μg for *Vespula*, 2.4–5 μg for *Dolichovespula* and 4.2–17 μg for *Polistes*) [63,64]. The size of insects from the *Vespa* genus would seem to indicate that a greater volume of venom is injected although there are no data to confirm this hypothesis. It has been reported that SCT results with *Vespula* spp. are less reliable than those obtained with *Apis mellifera*; this is because the amount of venom released by the vespids is more variable than injected by *Apis mellifera* [13]. Furthermore, vespids can sting several times and have the venom sac partially depleted whereas a bee only stings once and then dies from eventration. Differences have even been found within the same genus. In patients allergic to *Vespula* spp. with no VIT treatment, one study found a more aggressive attitude and a deeper sting with *Vespula maculifrons* which showed severe reactions than with *Vespula germanica* [12].

Other unknown aspects which affect the differences in the amount of venom inoculated are the time needed to empty the sac in a spontaneous sting, the different sizes of the insects, the possibility of having previously stung.

False safety and abandonment of VIT

After a tolerated SCT, VIT must continue following the same established protocol. It is important to bear in mind that a controlled sting does not replace a dose of VIT. The tolerated SCT confirms the success of the therapy and, therefore, that the therapeutic dose is effective. However, this success does not imply shortening the length of treatment (minimum of 5 consecutive years) [1]. Therefore, it is advisable that patients be informed that in spite of a negative SCT, they must complete the treatment with VIT to guarantee

its long-term efficacy [1]. Similarly, they must continue to carry an adrenaline auto-injector, if previously told to do so [65].

Discussion

In spite of the great advances in recent years in the diagnosis of allergies, currently no reliable biomarkers exist to monitor the efficacy of VIT. The SCT is the only reliable way of verifying the clinical efficacy of VIT. In real life and clinical research the SCT is accepted as the gold standard for measuring the protection provided by VIT [1], with doubts regarding the appropriateness of performing the test in untreated patients remaining due to the uncertainty surrounding the safety of the procedure [59,60].

The SCT methodology is not standardized. In a German publication [66] the SCT procedure has been described in the authors' care setting. The method adopted by Spanish allergists is based on the symbiosis with expert entomologists, along with the characteristics of the Spanish Health System and the experience accumulated over the last two decades.

This lack of standardization, which would include variations in the amount of venom injected, as well as the variability inherent in the different species of insects and their different geographic origin, may be related to the limited information which exists on the reproducibility of SCT results.

When faced with a patient who has experienced an anaphylactic reaction following a Hymenoptera sting without the normal diagnostic tools being able to confirm the underlying allergic mechanism, the SCT may be the best option to confirm the real need for VIT. However, confirmation of an isolated negative test does not rule out the

possibility of a systemic reaction following a subsequent sting [23,29,58] which means that the decision to start VIT should be taken with great care in these cases.

In treated patients the negative predictive value of SCT has not been explored so that always as far as possible it is necessary to perform more than one SCT to achieve a greater certainty regarding protection. However, the greater safety offered by the SCT in treated patients [21] allows more than one SCT to be performed under controlled conditions, thus adding a firm basis to the decision of discontinuing successful VIT. The incorporation of SCT into habitual clinical practice for patients allergic to Hymenoptera venom undergoing VIT guarantees an excellent level of integral care for these patients with it being clear that a negative result improves quality of life, and a positive result allows the patient to be reevaluated and improvements to be made to the VIT (protocol, dose, and duration of the same).

Regarding research, currently SCT is the necessary reference in the search for biomarkers of the effectiveness of VIT and immunological tolerance in general.

The main lights and shadows are summarized in table 3 and as an overall conclusion, the Hymenoptera allergy Committee of the SEAIC recommends using the SCT under appropriate conditions to assess the response to VIT, including the need for adjusting the dose and the appropriateness of maintaining treatment for more time and is in favor of more clinical multicenter studies using this technique being performed to improve the evidence base currently available. Furthermore, the Committee is not in favor of using the SCT for diagnostic purposes in untreated patients.

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Conflicts of Interest

The authors declare that have not conflict of interest to report.

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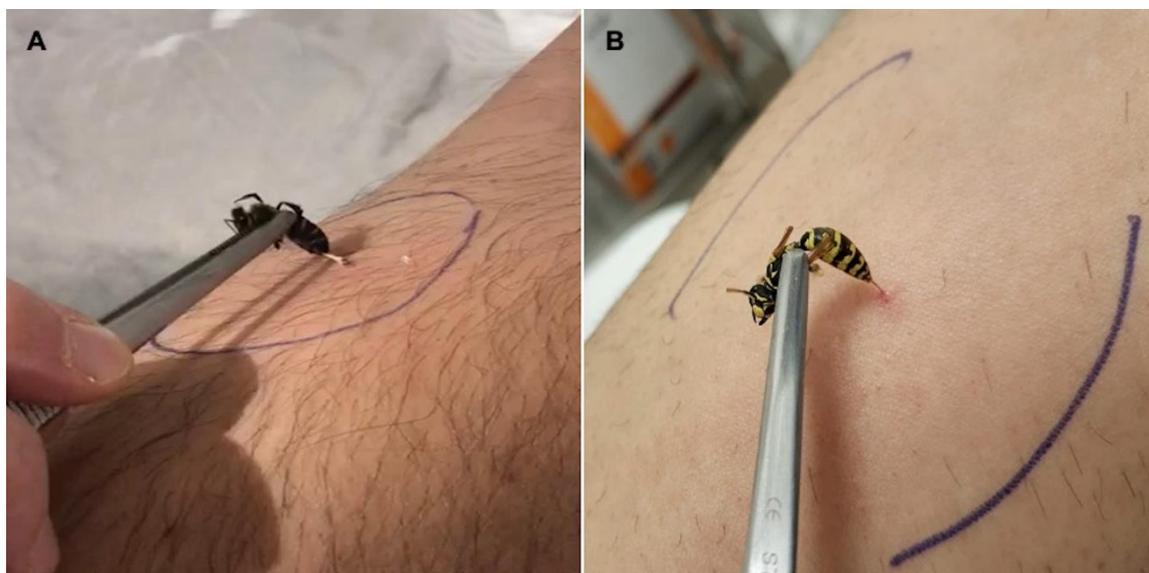
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FIGURES

Figure 1. The necessary material to perform Sting Challenge Test.



Figure 2. Sting Challenge Test with *Apis mellifera* (A) and *Polistes dominula* (B).



TABLES

Table 1. Indications and contraindications for the Sting Challenge Test.

Indications:
<ul style="list-style-type: none"> • Patients allergic to Hymenoptera venom in different phases of active treatment with VIT, with at least two months of good tolerance to treatment • To assess the decision to suspend this treatment (if the patient has had no spontaneous stings). • In the case of a reduction in quality of life as a result of uncertainty caused by the risk of a reaction due to a spontaneous sting. • Patients who have risk factors for the failure of treatment (those allergic to bee venom and those with systemic mastocytosis). • To verify that beekeepers and other high exposure professionals are protected before being re-exposed. • Patients who have discontinued VIT to evaluate the level of protection afforded by the years of treatment
Contraindications:
<ul style="list-style-type: none"> • Patients not receiving VIT treatment. • Patients treated with VIT who: <ul style="list-style-type: none"> - Have experienced a systemic reaction following a spontaneous sting. - Currently have systemic reactions with VIT - Have severe or uncontrolled cardiorespiratory disease (FEV1<70%), pregnant women or patients with acute inflammatory disease.

Table 2. Preparation of the room, material and staff

The test will be performed in the Allergy day hospital with availability of complete cardiopulmonary resuscitation equipment.
In patients at risk with comorbidities the room must have direct Access to ICU/Resuscitation room/Emergency room.
Direct supervision by an allergologist and nursing staff trained in emergency situations.
Material: <ul style="list-style-type: none">- Insects.- Transparent plastic containers with lids.- CO₂ cylinder with reducing valve and application pistol.- Standard dissection tweezers.- Entomologic scissors.
The test will last approximately 120 minutes.

Table 3. Summary of the lights and the shadows of the SCT.

The Sting Challenge Test	
The Lights	The Shadows
Possible biomarker Gold standard for efficacy of VIT	Debatable diagnostic tool
Programable procedure Tool for adjusting the dose of VIT	Unsafe in patients before VIT May be a scary experience for some patients
Safe in patients undergoing VIT Acceptable in patients with mastocytosis	Variability in amount of injected venom False feeling of safety
Useful for research	Complicated logistics of using live insects
Improves patients' quality of life	Non-standardized technique