

Skin testing technique and precision in stinging insect allergy

Massimo Corallino RN

Petty Officer of the Italian Navy, Nursing School of the Italian Navy, Taranto, and Chair of Allergology and Clinical Immunology, Medical Faculty and Nursing School, University of Bari, Bari, Italy

Andrea Nico MR

Chair of Allergology and Clinical Immunology, Medical Faculty and Nursing School, University of Bari, Bari, Italy

Georgios Kourtis MD

Chair of Allergology and Clinical Immunology, Medical Faculty and Nursing School, University of Bari, Bari, Italy

Maria Filomena Caiaffa MD, PhD

Associate Professor, Chair of Allergology and Clinical Immunology, Medical Faculty and Nursing School, University of Bari, Bari, and Chair of Allergology and Clinical Immunology, Medical Faculty and Nursing School, University of Foggia, Foggia, Italy

Luigi Macchia MD, PhD

Associate Professor, Chair of Allergology and Clinical Immunology, Medical Faculty and Nursing School, University of Bari, Bari, Italy

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Correspondence:

Dr Luigi Macchia

Chair of Allergology and Clinical

Immunology

Policlinico

70124 Bari

Italy

Telephone: 39 080 5478817

E-mail: l.macchia@allergy.uniba.it

CORALLINO M, NICO A, KOURTIS G, CAIAFFA MF & MACCHIA L
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Aim. We report on quantitative analysis of skin tests in patients undergoing *Hymenoptera* venom immunotherapy. The need for accuracy, coupled with a sound manual technique, in performing this procedure is emphasized. Involuntary errors may occur and pose serious problems with interpretation of results. A revealing example is reported and the strategy devised to analyse the flaws and overcome the resulting problems is presented and discussed.

Background. Skin testing plays a key role in the diagnosis of most allergic disease and in the assessment of allergen immunotherapy. Particularly, insect sting allergy requires implementation of complex and demanding skin testing protocols and a competent nursing practice.

Methods. Sixteen patients were tested before starting the immunotherapy and after three years of treatment. Cutaneous response (expected to decline, following immunotherapy) was assessed as: (i) allergen-elicited wheal areas; (ii) ratios between allergen-elicited wheal areas and homologous histamine (positive controls) wheal areas.

Results. By using allergen-elicited areas, the paradoxical result was obtained that skin reactivity had increased instead of decreasing, upon immunotherapy. Histamine response analysis suggested that this paradox might rather be the result of a technical flaw. Analysis of written notes of routine clinical meetings revealed that an important

manual flaw had been detected (and corrected) some years earlier, affecting the results of the baseline testing (viz. the allergen was injected deeper in the skin, yielding a weaker response). Skin reactivity evaluation in terms of allergen–histamine ratio confirmed this interpretation, as, when the baseline ratios were compared with the three years immunotherapy ratios, a distinct decline in skin reactivity was detected, as expected.

Conclusions. Skin testing in insect sting allergy is a conceptually and manually complex procedure, which should be subjected to systematic quality control assessment, like a laboratory procedure. The personnel involved in the performance of this procedure should receive appropriate and extensive training.

Relevance to clinical practice. Diagnosis of allergic diseases and monitoring of immunotherapy largely rely on impeccable skin testing technique.

Key words: allergy nursing, *in vivo* diagnostic procedures, nurses, quality control, skin testing, stinging insect allergy

Introduction

Insect sting allergy accounts for severe allergic reactions affecting an unknown but certainly not negligible number of people, worldwide. These reactions may sometimes be life threatening and cause death.

The stinging insects responsible for this severe form of allergy belong to the order of *Hymenoptera* and the vast majority of the cases are provoked by stings by yellow jackets (genus *Vespula*), wasps (genus *Polistes*), the domestic honey bee (*Apis mellifera*) and various hornet species (Mueller 1990, Golden 2003). High risk categories for stinging insect allergy comprise farmers, bee keepers, railway and highway workers, truck drivers, construction workers and, in general, people working or spending time outdoors, including the military. Allergic reactions to Hymenoptera stings can be regarded as a paradigm for all immunoglobulin E (IgE)-mediated reactions. Individuals with a genetic predisposition, referred to as atopy, may mount anomalous immunological responses to essentially harmless environmental antigens (such as proteins from pollen grains, animal danders and, as in this case, insect venoms), leading to production of substantial amounts of specific IgE antibodies. From the point of view of the organism immune defence capacity, these antibodies are of no meaningful use. Rather, by linking high affinity receptors on resident mast cells and circulating basophils, IgE antibodies couple the exquisite selectivity of the adaptive immune response to the devastating offensive potential of these specialized cells of the innate immunity and direct it against inappropriate targets. Thus, IgE-loaded mast cells and basophils will promptly release potent pro-inflammatory substances prepacked in their granules as well as newly synthesized mediators, as soon

as they come in contact with the specific antigen called, in this case an allergen, against which the IgE immune response has been mounted. As a result, allergic symptoms will arise, leading to asthma, hay fever, urticaria and other allergic conditions, including the life-threatening insect sting reactions (Austen 2001).

Risk of lethal events in subjects with a clinical history of anaphylaxis or high grade severity reactions upon hymenopteran sting can only be eliminated by allergen immunotherapy, consisting in desensitization by subcutaneous injections of the same venom responsible for the anaphylaxis reactions experienced previously (Golden & Schwartz 1986, Mueller 1990, Golden 2003). For several reasons, including prolonged duration (five years or more), inherent risk of anaphylaxis following immunotherapy administration and high costs, allergen immunotherapy demands elevated qualifications and excellent management and should be prescribed, administered and monitored by expert medical and nursing personnel.

One particularly important problem in stinging insect allergy is represented by the accuracy and precision of allergic skin testing. Skin tests are essential for the diagnosis of all allergic conditions (Demoly *et al.* 2003) but are even more important in insect sting allergy (Hunt *et al.* 1976, Biló *et al.* 2005). In fact, the precise identification of the responsible Hymenoptera and, hence, the selection of the correct venom for allergen immunotherapy, to a large extent, rely on skin test procedures, as venom-specific IgE are often found at rather low levels in the serum (detectable by radioallergo-sorbent test; Sobotka *et al.* 1978, Golden 2003). Moreover, monitoring of skin reactivity to venom allergens, attained by means of impeccably performed quantitative skin tests, alongside monitoring of changes in venom-specific serum

IgE and IgG levels, is one of the few tools for assessing the biological effects of the immunotherapy (Thurnheer *et al.* 1983, Golden *et al.* 1992, Golden 2003) and, therefore, for inferring on the degree of clinical protection achieved. Important decisions, including discontinuation of immunotherapy, are based on skin testing results (Thurnheer *et al.* 1983, Golden *et al.* 1998). For these reasons, skin tests in stinging insect allergy are often performed by using two distinct techniques in combination with each other: skin prick testing and intradermal testing. Moreover, serially increasing concentrations of allergens are usually assayed, particularly in intradermal testing.

In the highly standardized technique of skin prick testing the skin (usually the volar forearm surface) is punctured through a droplet of *Hymenoptera* venom protein solution, typically at a concentration of 100 µg/ml (sometimes stepwise incremental concentrations are also used), by means of a disposable hypodermic needle or (more often) other specifically developed prick needles. In the much less standardized intradermal testing, instead, 0.05 ml of serial 10-fold increasing concentrations of venom extracts are gently injected at exactly the border between epidermis and dermis. A tuberculin syringe is used with a 26- to 27-gauge needle, penetrating the skin at a 10–12° angle. Typically, 10 000- to 100-fold dilutions of the extract used for skin prick testing are employed for intradermal testing (Demoly *et al.* 2003). Thus, if a 100 µg/ml solution is used for skin prick testing, 0.01, 0.1 and 1 µg/ml solutions of the test venom, respectively, may be used for carrying out the intradermal testing. Skin prick tests and intradermal tests, at various allergen concentrations, are usually carried out consecutively in one session, as a multi-step procedure, with any single step requiring 20–30 minutes for the skin response to become apparent and be recorded on a registration sheet, to allow convenient quantitative evaluation and analysis. Moreover, several venoms are usually tested in the same session (e.g. four in our protocol). Positive responses consist in a wheal-and-flare reactions, whose magnitude essentially reflects the amount of endogenous histamine released by activated cutaneous mast cells. In turn, the magnitude of mast cell activation essentially reflects the amount of the existing specific IgE attached to their surface by high affinity receptors (IgE cross-linking by allergen molecules penetrating into the skin triggers mast cell activation and histamine release). In quantitative skin testing, to measure the elicited cutaneous reactions, the wheal can be sharply outlined with a felt-tip pen and the outline can be blotted on removable tape and recorded on paper, for convenience. The average diameter of wheal is measured, expressing the magnitude of the positive

response. Alternatively (and preferably) the area of the wheal can be calculated, representing an even more precise way of quantitative assessment (Østerballe & Weeke 1979, Dreborg 1993, 2001). Therefore, it does not need to be underlined that quantitative skin testing in insect sting allergy is a complex and prolonged procedure, which requires skill, precision and accuracy.

Here we report on the quantitative analysis of skin tests carried out in 16 patients undergoing allergen immunotherapy for *Hymenoptera* venoms, at two different times, three years apart, in order to assess the biological effects of the treatment, typically resulting (also) in a decrease in specific skin reactivity (Thurnheer *et al.* 1983, Golden 2003), which, in turn, depends in a large proportion on the decrease in specific IgE being produced against the immunotherapy venom.

This study emphasizes the absolute need for accuracy, coupled with a sound manual technique, in performing this procedure. Involuntary imperfections may occur at any time and cause serious problems with the interpretation of results. A revealing example is reported and the strategy we devised to analyse the flaws and overcome the resulting problems is presented and discussed.

Methods

Study design

This is a descriptive study, which analyses the results of quantitative skin testing with insect venom allergens in a series of 16 consecutive patients with severe stinging insect allergy, subjected to allergen immunotherapy. The tests were performed at two different time-points, i.e. at diagnosis, before starting the immunotherapy (baseline) and approximately three years later (time-point 3 y). Cutaneous response to the allergen used in immunotherapy is expected to decline over time, as the treatment proceeds, and may provide valuable information on the immunological effects of the treatment (Thurnheer *et al.* 1983, Golden 2003). Primary data, i.e. the blotted outlines of the wheals generated by the skin tests performed at the two time-points indicated, were originally collected and analysed with the sole purpose of assessing the extent of this decline. However, recognition of apparently paradoxical results led us to investigate the possibility of technical flaws, underlying these unexpected data. The records of the clinical activity carried out with these patients over a rather prolonged period were accurately examined and an explanation for the faulty results was identified. Moreover, an alternative computation method for skin test response, i.e. expressing the test outcome in terms of ratio between allergen

wheel area and histamine wheel area (referred to as a skin index; see below; Macchia *et al.* 1991), rather than wheel area only, was adopted, allowing unambiguous interpretation of the global data and suggesting that indeed an unnoticed, yet amendable, error in the manual execution of the intradermal test had repeatedly occurred several years before the time when the analysis was carried out.

Participants

Sixteen patients with a clinical history of anaphylaxis upon insect stinging were diagnosed with severe stinging insect allergy during the year 2001 and prescribed allergen immunotherapy, with relation to the high risk of further anaphylaxis reactions. Of them, 15 were males and one was female; their mean age at diagnosis was 39.9 years (SD 15.5).

Quantitative skin testing

Skin tests were performed by two distinct techniques: skin prick testing and intradermal testing. Both techniques were carried out in a single session, sequentially in a simplified three-step procedure. Thus, the patients were first subjected to skin prick tests and successively to intradermal tests. Moreover, the intradermal tests were executed with two different, 10-fold allergen concentrations. The lowest concentration was tested first, followed by the highest allergen concentration.

Insect allergens and histamine solution

Four insect allergens were tested at baseline, reflecting the epidemiology of stinging insect allergy in the geographic area where the patients resided. These four allergens were: *Vespula* spp., *Polistes* spp., *A. mellifera* and *Vespa crabro* (the European hornet). At time-point 3 y, only the allergen used for immunotherapy was tested (in order to detect a possible decline in skin reactivity). Lyophilized *Vespula* spp., *Polistes* spp. and *A. mellifera* venoms were supplied by Dome-Holister-Stier Miles, Spokane, WA, USA, and reconstituted at the appropriate concentrations in 1% albumin saline. Only freshly prepared solutions were used. *Vespa crabro* venom was supplied by Anallergo, Florence, Italy, as a ready-to-use solution. As none of 16 patients of this series was diagnosed with *V. crabro* allergy, this allergen was never considered at time-point 3 y (and will be no longer mentioned). Histamine hydrochloride 10 mg/ml (histamine base) in 50% glycerol solution (from Stallergenes, Antony, France) was used as the positive control in skin prick testing. A 0.002 mg/ml watery solution of the same reagent was used as the positive control in intradermal testing. Saline with 1% albumin served as the

negative control in both skin prick testing and intradermal testing.

Skin prick testing

The tests were performed on the volar surface of the forearm by puncturing the skin through a droplet of a 100 µg/ml allergen with an Østerballe and Weeke-type 1 mm point length standardized needle (Østerballe & Weeke 1979), supplied by Stallergenes. The pricker was inserted perpendicularly into the skin with a moderate pressure. Positive and negative control solutions were tested in the same manner. Results were read 20 minutes after pricking.

Intradermal testing

These tests also were performed at the level of the volar forearm skin. Thus, 0.05 ml of allergen solutions were gently injected by a 26-gauge needle tuberculin syringe at the interface between epidermis and dermis. The needle penetrated the skin with an angle of 10–12° and with the sloping part of the needle tip facing up. When accurately performed the injection produced a lentil-like epidermis swelling, approximately 4–5 mm in diameter. As already mentioned, two 10-fold allergen concentrations were used in our protocol: 0.1 and 1 µg/ml, in 1% albumin saline, corresponding to 1000- and 100-fold dilutions of the solution used for the skin prick tests, respectively. The 0.1 µg/ml concentration was tested first and the results were read 20 minutes after injection. Then, the 1 µg/ml concentration was tested and, again, the results were read after 20 minutes. When a wheal with a mean diameter exceeding 10 mm was generated by intradermal test with the 0.1 µg/ml solution of one of the allergens, the test with the 1 µg/ml solution of that allergen was omitted, to minimize the risk of large local or systemic reactions. Histamine hydrochloride 0.002 mg/ml and the allergen solvent were injected at the 0.1 µg/ml allergen solution step, as the positive and the negative control, respectively.

Skin response recording and quantitative assessment

The wheals generated by skin prick tests and intradermal tests were precisely outlined with a felt-tip pen and the outline was blotted to a transparent removable tape and the tape pasted on a record sheet. The mean wheal diameters were calculated at the end of the skin testing session and used for immediate decision making (e.g. diagnosis, therapy, etc.) and for the patient result sheet. [The mean diameter is defined as the average between the maximum possible wheal diameter and the diameter orthogonal at midpoint of the maximum diameter (Dreborg 1993).] Obviously, skin testing wheals often tend to have an irregular shape. The wheal areas,

including those of the baseline skin testing session, were assessed only after performing the point-time 3 y skin test session (when a comparison between results at the two time-points was sought). The area was calculated by superimposing a transparent cellulose acetate sheet with a 1 mm square lattice grid and counting the squares comprised within the outline (each square 1 mm²).

Skin index is defined as the ratio between the allergen wheal area and the homologous histamine wheal area (in this case, 10 mg/ml histamine for skin prick tests and 0.002 mg/ml histamine, for intradermal tests; see Macchia *et al.* 1991).

Statistics

Cumulative results are expressed in terms of arithmetical mean values with standard deviations, as analysis by the Kolmogorov–Smirnov test revealed that the data examined were normally distributed. The Student's *t*-test for paired data was used to analyse the significance of the differences between the baseline and the point-time 3 y mean values.

Ethics

As mentioned, the data analysed here were generated as part of a normal immunotherapy monitoring process, according to the protocol in use at our unit, of which patients had been adequately informed. However, when we decided to present the data collected in this paper, we asked the patients in the study for their formal informed consent, in observance of the Declaration of Helsinki. The approval of the ethics committee of one of the institutions involved in the study (University of Foggia) was sought and obtained.

Results

Specific skin reactivity assessment in terms of wheal areas

Skin reactivity to specific *Hymenoptera* venom allergens was assessed in 16 patients undergoing allergen immunotherapy, at two different time-points: immediately before the immunotherapy onset (baseline) and approximately three years later (36.5 months, on average; SD = 5.4), halfway into the five-year treatment (time-point 3 y). Of the 16 patients, 11 received immunotherapy with *Vespula* venom; four received immunotherapy with *Polistes* venom and three immunotherapy with *A. mellifera* venom (in two cases a double immunotherapy course, with both *Vespula* and *Polistes* venoms, was undertaken).

The results obtained by measuring the areas of the wheals generated in these patients by the skin prick test technique and the intradermal test technique, performed with 0.1 and 1 µg/ml venom solutions, respectively, are shown in Table 1a and Fig. 1a–c (*Vespula* data only).

As it can be seen, when the results obtained by the skin prick test technique were considered, only minor changes in skin reactivity were appreciated at time-point 3 y, when compared with baseline (Table 1 and Fig. 1a).

In contrast, when the wheal areas generated by intradermal test technique with specific allergens at 0.1 µg/ml concentration were examined, it was found that, apparently, the specific skin reactivity had undergone a substantial increase after three years of immunotherapy (Table 1a and Fig. 1b). Thus, specific skin reactivity for the three venom allergens considered appeared to have increased by approximately 35% in the case of *Vespula* and approximately twofold in the case of *Polistes* and *A. mellifera*.

Table 1 Results of quantitative skin tests performed immediately before starting allergen immunotherapy (Baseline) and after approximately three years of continuous treatment, respectively, in 16 patients with *Hymenoptera* venom allergy. (a) Results expressed as the average area of the wheals, with standard deviation (mm²). (b) Results expressed as the average skin index (i.e. the ratio between the area of the allergen-elicited wheal and the area of the homologous histamine-elicited wheal), with standard deviation

	SPT (100 µg/ml)		IDT (0.1 µg/ml)		IDT (1 µg/ml)	
	Baseline	3 years	Baseline	3 years	Baseline	3 years
(a)						
<i>Vespula</i> , n = 11	5.25 SD 6.63	5.07 SD 5.74	24.86 SD 31.31	33.79 SD 39.78	28.78 SD 23.62	48.30 SD 44.91
<i>Polistes</i> , n = 4	4.63 SD 5.34	2.48 SD 4.37	9.5 SD 11.15	21.83 SD 37.82	24.75 SD 20.09	24.30 SD 46.17
<i>Apis mellifera</i> , n = 3	4.67 SD 4.51	4.27 SD 3.70	29 SD 14	58.67 SD 35.30	16.5	37.5
(b)						
<i>Vespula</i> , n = 11	0.25 SD 0.30	0.22 SD 0.22	1.69 SD 2.30	0.72 SD 0.75	1.32 SD 1.21	0.91 SD 0.76
<i>Polistes</i> , n = 4	0.18 SD 0.20	0.15 SD 0.26	0.55 SD 0.51	0.36 SD 0.62	1.89 SD 1.92	0.49 SD 0.72
<i>Apis mellifera</i> , n = 3	0.46 SD 0.58	0.21 SD 0.20	2.37 SD 1.90	0.82 SD 0.70	1.27	0.29

Note that two patients were diagnosed as being allergic to both *Vespula* and *Polistes* venoms and were given immunotherapy for both allergens. SPT, skin prick tests; IDT, intradermal tests.

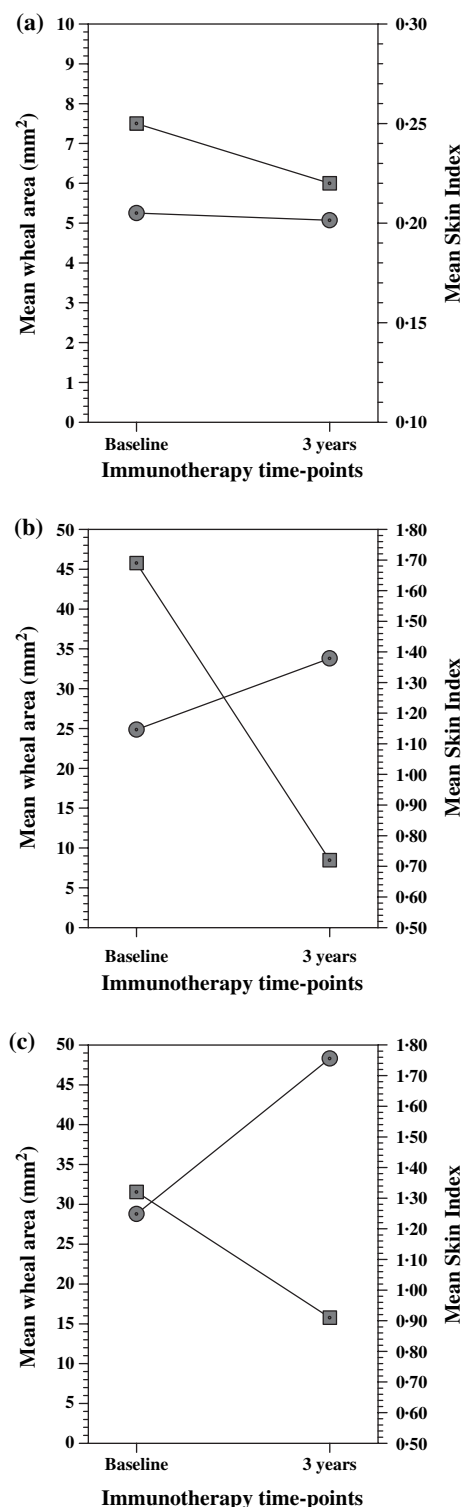


Figure 1 Changes in specific skin reactivity in 11 patients with severe allergy to *Vespula* venom and undergoing allergen immunotherapy with the same venom. Skin reactivity was assessed before starting the treatment (Baseline) and three years into the treatment (3 years), which usually lasts for five years or more. Circles are used for skin reactivity expressed in terms of mean wheal area (mm²); squares represent skin reactivity expressed in terms of average skin index (i.e. the ratio between the area of the allergen-elicited wheal and the area of the homologous histamine-elicited wheal). (a) Results obtained by skin prick tests, performed with the allergen at a concentration of 100 µg/ml. Histamine concentration was 10 mg/ml. (b, c) Results obtained by intradermal tests, performed with the allergen at concentrations of 0.1 and 1 µg/ml, respectively. Histamine concentration was 0.002 mg/ml.

increase (Table 1a and Fig. 1c), while *Polistes* skin reactivity had remained unchanged.

Retrospective detection of skin testing technique mistakes

These obviously paradoxical results, in sharp contrast to the expected decline in specific skin reactivity upon an allergen immunotherapy course conducted for three years (Thurnheer *et al.* 1983, Golden 2003), led us to analyse the areas of the histamine controls obtained at the two time-points with the skin prick testing technique and the intradermal testing technique, respectively. [One should expect that the non-specific, exogenous histamine-triggered skin reactivity will remain essentially unchanged when the test is performed in the same group of subjects on two different occasions (Dreborg 2001).] Thus, the average area of the histamine (10 mg/ml) skin prick test control wheals was 21.35 SD 9.09 mm² at baseline and 29.16 SD 14.64 mm² at time-point 3 y (the difference was found to be statistically non-significant when analysed by the Student's *t*-test for paired data, *p* = 0.073). In contrast, the average wheal areas generated by intradermal injection of histamine (0.05 ml of 0.002 mg/ml solution) at the two time-points were 22.21 SD 16.99 and 64 SD 44.89 mm², respectively (in this case the Student's *t*-test for paired data indicated that the observed difference was statistically significant; *p* = 0.012). This further observation led us to consider the possibility that the detected increase in skin reactivity might in fact have been due to some kind of unintentional systematic error in the intradermal skin testing technique. That prompted a thorough retrospective examination of the files relevant to the 16 cases considered. Moreover, the records of the weekly meetings of the clinical team in charge for diagnosing and treating insect allergy were carefully reviewed.

In fact, it was found that in July 2002 it had been noticed that not only the size of the wheals relevant to intradermal

Accordingly, the results of the intradermal tests performed with 1 µg/ml solutions of purified *Vespula* venom and *A. mellifera* venom also apparently indicated that skin reactivity at time-point 3 y had undergone a more than twofold

tests performed with venom allergens but also that of intradermal tests performed with histamine 0.002 mg/ml (positive controls) were 'unusually and inexplicably small'. At first, the possibility that the histamine solution in use at that time, for some reason, had a lower than expected activity *in vivo* was investigated, by performing intradermal tests in five non-allergic volunteers compared with a freshly prepared solution of histamine hydrochloride (obtained from Sigma, St Louis, MO, USA; both solutions had the same concentration of 0.002 mg/ml). Based on the results of these tests (data not shown), the hypothesis of a weak skin response due to a defective histamine solution had been ruled out.

It had been then realized, also by accurate and direct reviewing of the actual intradermal skin testing procedure, that the technique itself, as performed at that time by the personnel in charge (both nurses and medical residents), had slowly and deceptively become imperfect: histamine as well as allergen solutions were injected slightly deeper than exactly at the interface between epidermis and dermis, thus generating wheals sizeably smaller when compared with those elicited by injecting the same amount of histamine or allergen in the correct manner. It had also been possible to establish that the period of time when intradermal testing might have been ridden by these procedural flaw had gone from the end of 2000 to July 2002 (hence, spanning the entire 2001, when the baseline tests in the 16 study patients had been performed).

Specific skin reactivity assessment in terms of skin index

Analysis of the above circumstances made us reckon that, perhaps, a faulty manual technique could have caused the intradermal testing results at baseline to be considerably underestimated, in some, at least, of the 16 study patients. This conclusion offered an adequate explanation for the observed increase in specific skin reactivity after immunotherapy (as opposed to the expected decrease), effectively freeing us from the need of elaborating on other obscure as well as improbable biological interpretations.

Therefore, we re-analysed the skin reactivity at the two time-points considered by using the skin index (Macchia *et al.* 1991), i.e. the ratio between the area of the allergen-elicited wheal (a venom allergen in this case) and the area of the histamine (0.002 mg/ml) control wheal. This method of skin testing result assessment in allergic diseases can be used in order to adjust for any variations in non-specific skin reactivity, when comparing results of tests performed in different subjects or in the same subject at different time-points (as in this case). Moreover, this method allows correction of systematic errors, including skin testing

technique errors, as a given error will affect both the allergen wheal size and the histamine wheal size in the same fashion.

In fact, implementation of this approach in the analysis of results obtained by intradermal testing, with both concentrations used (i.e. 0.1 and 1 µg/ml), revealed that at time-point 3 y there was a considerable and consistent decline in skin reactivity, when compared with baseline, for all three allergens considered (see Table 1b). In particular, at time-point 3 y, in the patients who received *Vespula* immunotherapy the 0.1 µg/ml and the 1 µg/ml intradermal test skin indexes underwent an approximate 55% and 30% decline, respectively, as expected (Fig. 1b–c). In addition, further analysis of skin prick test results by the skin index method consistently confirmed the decline in skin reactivity already observed with the intradermal tests (Table 1b and Fig. 1a).

Statistical analysis

Probably because of the small sample size of the data analysed, none of the differences in skin reactivity listed in Table 1a,b was found statistically significant, according to the Student's *t*-test for paired data. Thus, *p*-values ranged from 0.094 to 0.988, with the exception of comparison between wheal areas of intradermal tests performed with 1 µg/ml *Vespula* venom, which yielded a *p*-value almost approaching significance: 0.052. Nevertheless, considering the consistency of the results, particularly those in Table 1b, we regard these data as reliable and trustworthy.

Discussion

Skin testing is a fundamental procedure in the diagnosis of most allergic conditions. Moreover, skin testing plays a key role in allergen immunotherapy assessment as well as in surveys dealing with the epidemiology of allergic diseases (Demoly *et al.* 2003). Strict adherence to standardization rules and methodological accuracy, performing the test in a quantitative way and expressing their results also quantitatively, in terms of wheal mean diameter or, better, in terms of wheal area, have become increasingly recommended (Dreborg 2001) and implemented at the level of the best allergology practice.

This is particularly true with insect sting allergy, both at the diagnosis and at the immunotherapy assessment level, for several reasons, including frequent severity of symptoms, low level of circulating IgE (Sobotka *et al.* 1978, Golden 2003, Biló *et al.* 2005), lack of feedback from periodic exposure to the allergen (as it happens for pollen-related allergic diseases,

for example), special caution with decision making on immunotherapy discontinuation (Thurnheer *et al.* 1983, Golden *et al.* 1998). However, skin testing in insect sting allergy is a particularly complex and demanding procedure, requiring competent nursing practice, when, as in our setting, four venom allergens are tested simultaneously in a multi-step procedure based on two different skin testing techniques (skin prick tests and intradermal tests), of which one is performed with two different allergen concentrations (three concentrations in other settings). Due to this complexity, this procedure is inherently prone to mistakes and should be subjected to continuous and strict quality control as if it were a laboratory method, rather than a clinical procedure (Dreborg 2001). However, mistakes may occur, the more dangerous being those that become eventually established because of subtle, imperceptible, day-by-day decline in accuracy. It is this kind of mistakes that are more likely to put in jeopardy the results of this most important allergology procedure.

Here we report and comment on a particularly clear and instructive case. As it was appreciated by the analysis of the written notes made on occasion of routine weekly clinical meetings, in July 2002 we had detected (and corrected) an important flaw in intradermal test performance, viz. the allergen was being injected too deep in the skin, yielding a considerable decrease in the magnitude of the skin response and, consequently, a possible decrease in the method sensitivity.

Moreover, this flaw would have had unforeseen consequences on the assessment of the immunological response to immunotherapy in a group of 16 patients subjected to this treatment, as it was understood about three years later, when another round of skin tests was performed. This time by using a flawless manual technique. Thus, when the baseline skin response was compared with the time-point 3 y response, the paradoxical result was obtained that the skin reactivity had undergone an increase in association with immunotherapy rather than a decrease, as expected. Analysis of histamine reactivity, at baseline and at time-point 3 y, respectively, immediately suggested that this paradox should have been the result of a technique mistake, rather than the expression of an improbable biological phenomenon. In fact, while histamine skin response as estimated by skin prick testing remained essentially unchanged, histamine skin response elicited by intradermal tests underwent an almost threefold and statistically significant increment, from 22.21 to 64 mm², on average. The possibility of access to the 2002 notes led us to rapidly realize that indeed this was the case. This hypothesis was further proved when skin reactivity was evaluated in terms of skin index, which takes into account also the histamine

response in denoting the magnitude of the skin response to an allergen. Thus, when the baseline average indexes were compared with the average skin indexes at time-point 3 y, a distinct decrease was detected, as expected. As already noted, we think that failure by the statistical test used to show that the differences calculated, as reported in Table 1b, were statistically significant does not preclude this interpretation, given: (i) the rationale that if a technical error had occurred (and we knew it had) its consequences should have had bearing on both allergen wheals and histamine wheals, and (ii) the definite consistency of results.

As an additional observation, from Table 1b and Fig. 1a it can be also noticed that, by using the skin index approach instead of the simple indication of wheal areas, the comparison of skin reactivity obtained by the skin prick test technique at the two time-points clearly indicates a decline after three years of treatment, in line with the results of the intradermal tests. These observations suggest that the skin index, although difficult to use in the everyday allergology practice, may represent a tool for fine tuning the skin testing results, when applied to research work or, as in this case, to data that should be subjected to accurate analysis at a later time and are not required for immediate decision.

Finally, it is noteworthy that the magnitude of the skin response elicited by the skin prick tests was much lower when compared with that elicited by the intradermal tests (Table 1a and Fig. 1a–c), while the skin indexes relevant to the skin prick tests were consistently below 0.5 (Table 1b; i.e. on average, the cutaneous response elicited by the skin prick tests was more than 50% less intensive when compared with the response due to the histamine control; in other words it was rather weak). These data add to the notion that in insect venom allergy using skin prick tests only would imply the risk of false-negative results, due to the low sensitivity of the method. On the other hand, performing the skin prick tests may in any case be worthwhile, representing a kind of 'in test' validation of the intradermal tests, which, in turn, may sometimes pose problems of specificity (Demoly *et al.* 2003).

In conclusion, there are several lessons that either we were reminded of or we learned anew from the analysis of the circumstances and the data reported above and that can be of general interest for nurses as well as medical personnel involved in diagnosing and treating allergic diseases. We would like to summarize these lessons as follows:

- 1 Skin testing in allergology is a conceptually and manually complex procedure, which requires skill, patience, attention and precision (Dreborg 2001). This is particularly true when applied to insect sting allergy. The personnel involved in the execution of this procedure should receive appropriate and extensive training. In particular, it would

be desirable that nurses with special competence in the field of allergology are prepared through specific education (as more and more it is happening). Skin tests are likely to provide fast, abundant and accurate information (Sibbald *et al.* 1997, Demoly *et al.* 2003) but only when performed at the level of an excellent clinical and nursing practice. Misleading results may otherwise be obtained, as in the case described.

- 2 When facing a fresh and demanding challenge, such as solving a puzzling, clinical or technical problem, devising a new protocol or modifying an established one, a well-planned, easy-to-perform and readily informative pilot study may be carried out to put things on the right track timely.
- 3 Organize working parties, in which manual procedures requiring particular skill and precision, as for the intradermal testing or skin prick testing, are performed and reviewed by senior personnel. These working parties should be held on a regular basis. We propose at least twice a year.
- 4 Always express the results of skin testing in a quantitative way, in terms of wheal average diameter or, preferably, in terms of wheal area (Malling 1984, Dreborg 2001).
- 5 Pay particular attention to the magnitude of the histamine response. It is the histamine response that validates the tests (Malling 1984, Dreborg 2001).
- 6 When facing unexpected or otherwise anomalous results, calculate the skin index and use the information it provides in association with that deriving from the sheer quantitative assessment of skin response to allergens.
- 7 Hold weekly clinical meetings, where the problems of the everyday activity are raised and discussed and where solutions are found. Make sure that all personnel are involved, doctors and nurses alike. Always make written notes; save the records and, most importantly, remember that there may be notes useful to read and read them, when needed.

Contributions

Study design: LM, MFC; data analysis: MC, AN, GK, LM; manuscript preparation: MC, LM.

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