Diplomarbeit

Intradermal Testing in Hymenoptera Venom Allergy

eingereicht von

Bernhard Strohmeier

Mat.Nr.: 0312949

zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde (Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an der

Universitätsklinik für Dermatologie und Venerologie Graz

unter der Anleitung von

Priv.-Doz. Dr. Gunter Sturm

Graz, 22.04.2010 (Unterschrift)

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe. Die benutzten Quellen sind im Kapitel References detailliert, vollständig und nachvollziehbar aufgelistet.

Graz, 22.04.2010 (Unterschrift)

Acknowledgment

Hereby I want to express my gratitude to all those people who contributed in different ways to this work.

My sincere thank goes to Priv.-Doz. Dr. Gunter Sturm for his excellent supervision and mentoring throughout the entire thesis. It was a pleasure to work with him.

I want to express my immense gratitude to my brother Dipl.-Ing. Tobias Strohmeier, who helped me with the data analysis.

Furthermore I want to thank my parents for the unwavering support during the entire study period.

Abstract

Introduction: According to current guidelines intradermal testing with Hymenoptera venom should be performed stepwise with 15-20 min breaks after each injection of venom. In this study, we administered different venom doses during intradermal testing simultaneously to save time for the diagnostic procedure. The objective of the present study was to determine the frequency of adverse reactions of simultaneous intradermal testing.

Patients and Methods: Simultaneous intradermal testing with concentrations of 0.00001, 0.0001, 0.001, 0.01, 0.1, and 1 μg/ml Hymenoptera venom was performed in 484 patients who experienced anaphylactic reactions after a Hymenoptera sting. An intravenous line was inserted before testing. The respective test protocols and documented adverse reactions were collected and analyzed.

Results: 6 of 484 (1.2 %) patients suffered from side effects during simultaneous intradermal testing: 2 showed symptoms of vasovagal disorder after insertion of the intravenous line; 3 subjects felt dizziness, sensation of heat and dyspnea during the test; only one patient suffered from a clear anaphylactic reaction with red flush on the site of the test, shivering and nausea, but without tachycardia or hypotension.

Conclusion: Simultaneous intradermal testing is time-saving and safe in the diagnosis of Hymenoptera venom allergy. Large prospective studies are needed to evaluate the frequency of side effects during simultaneous intradermal testing.

Zusammenfassung

Hintergrund: In den aktuellen Richtlinien zur intradermalen Hauttestung mit Insektengift wird eine stufenweise Verabreichung mit 15-20 min Wartezeit zwischen den einzelnen Testkonzentrationen empfohlen. Das Ziel der vorliegenden Studie ist die Evaluierung der Nebenwirkungen bei gleichzeitiger intradermaler Verabreichung aller Testkonzentrationen.

Patienten und Methoden: Bei 484 PatientInnen mit einer anamnestisch erhobenen anaphylaktischen Reaktion nach einem Hymenopterastich wurden simultane Hauttestungen durchgeführt. Alle Testkonzentrationen (0,00001, 0,0001, 0,001, 0,01, 0,1, 1 μg/ml) wurden gleichzeitig intradermal injiziert. Aus Sicherheitsgründen wurde vor jeder Testung ein intravenöser Zugang gelegt. Die jeweiligen Testprotokolle wurden hinsichtlich unerwünschter Nebenwirkungen ausgewertet.

Ergebnisse: Bei 6 (1,2 %) von 484 PatientInnen waren Nebenwirkungen während der Testung objektivierbar: 2 zeigten vasovagale Symptome nach Anlegen des intravenösen Zugangs; 3 gaben Hitzegefühl, Schwindel und Atemnot nach Verabreichung der Testkonzentrationen an; nur 1 Patientin zeigte eindeutige allergische Symptome während des Hauttests: flächige Rötung am getesteten Arm, Schüttelfrost, Übelkeit, jedoch keine Tachykardie oder Hypotension.

Schlussfolgerung: Simultane intradermale Hauttestung ermöglicht eine zeitsparende und dennoch sichere Diagnostik der Insektengiftallergie. Prospektive Studien mit einer großen Anzahl von PatientInnen sind zur genauen Evaluierung der Häufigkeit von Nebenwirkungen notwendig.

Table of Contents

1) Introduction

The majority of insect stings induce a transient local reaction, which usually resolves after hours/days without any treatment. The venom of some species of Hymenoptera can provoke a systemic allergic reaction in sensitized humans. It is estimated that potentially life-threatening systemic reactions occur in about 2 % of the general population.

Intradermal skin testing in Hymenoptera venom allergy is a useful diagnostic tool. If performed stepwise under current guidelines it is a very time-consuming procedure for the medical staff and for the patient.

The advantages and disadvantages of simultaneous intradermal skin testing with Hymenoptera venom have not been evaluated yet. The aim of this study was to answer the following questions:

- What is the frequency of adverse reactions in simultaneous intradermal skin testing?
- Is there an increased risk for systemic anaphylactic reactions during simultaneous intradermal testing compared to other diagnostic procedures?

Additionally the background of Hymenoptera venom allergy with a focus on current diagnostic procedures is described in a detailed review.

2) Taxonomy of Hymenoptera

This taxonomy is based on the book *Bees, Wasps, Ants* (1), which belongs to the core literature in the German-speaking part.

Hymenoptera are widespread over the whole earth and one of the largest orders of insects, including bees (Apidae), wasps (Vespidae) and ants (Formicidae). The name refers to the special construction of the transparent, shimmering wings. The body is separated into three main parts: head, thorax and abdomen. Each part is characteristic for one of more than 115,000 species. In general, a correct assignment to a specific family can only be done under the microscope. In medicine, thanks to similar composition of venom, it is sufficient to divide into the two families Apidae and Vespidae, who both belong to the suborder Aculeata. About 10 % of the world's Hymenoptera population is present in Europe.

Aculeatae are highly developed social insects and live in networks of different classes. Up to now approximately 1000 species were defined. The constricted waist in the lower segment is the common sign of this suborder. Humans are mainly confronted with sterile female workers, who are responsible for brood care, nest building and defense. The circle of life of the working class is 4 to 5 weeks. In female workers, the ovipositor, usually a device to deposit eggs is modified to a stinger. With this tool the venom is transmitted into the human subcutaneous tissue. The surface of the lancet is covered with barbs, especially in bees. This is the reason why the stinger of bees, often with attached venom sac, usually remains in the elastic human. A retained stinger is a hint, but not a proof for the bee as the responsible insect for a sting reaction. Bumble bees and Vespidae usually do not leave a stinger, but of course exceptions are possible (2). The exact composition of different Hymenoptera venoms is described below.

Depending on the species and climatic conditions, the active time of Hymenoptera – and therefore the highest risk to get stung – is from the

beginning of March to the end of October. Especially on warm and sunny days a remarkable number of female workers leaves the hive to collect food. A peak of activity can be noticed in June and July. In very warm summers a second peak, after reduced activity in August, occurs in September, when an additional generation is born. The most risky daytime for patients with an allergic reaction in history is from 11 a.m. to 5 p.m., when the temperature reaches its maximum.

2.1) Apidae

The family Apidae is represented in middle Europe by the honeybees (Apis mellifera, belonging to the subfamily Apinae) and the bigger bumble bees (subfamily Bombinae).

Figure 1

Honeybees are of uniform brown color, while bumble bees have very characteristic white and yellow bands on the abdomen (see figure 1). In distinction to Vespidae they have hairs on their trunk. Gardeners value them as very effective pollinators in glasshouses, especially the extensive hairy bumble bee. While honeybees live in huge hives (up to 80.000 insects are possible), Bombinae are quite rare and form only small networks of not more than 200 individuals.

Like all Hymenoptera, Apidae prefer moderate to warm climate. Nevertheless, owing to their hairy surface and the compact body, bumble bees are able to live under quite cold circumstances, like in alpine destinations and the northern part of Europe. On the other hand, Bombinae show rare occurrence in tropical and subtropical countries. Around September, with the death of the old queen, the decline of the whole bumble bee hive starts. Only the young queens survive and are able to found a new network in springtime.

In comparison to Vespidae, Apidae behave fairly peaceful and tend to attack only after provocation like overpassing the critical distance to the nest. In general the whole beehive survives through winter, for this reason a noticeable amount of honey bees already exists in springtime. Honeybees produce in the inside of their nest temperatures that can be much higher than in the surrounding environment. Therefore even in wintertime female workers, after warming up in the hive, are able to fly and sting. Doctors have to take this fact into account, especially at the beginning of a venom immunotherapy. Also in winter patients should be requested to close the window and not go outside during the start of therapy.

2.2) Vespidae

Although the venom of Vespidae is very similar within the family, the taxonomy is quite difficult. As a common sign all Hymenoptera belonging to Vespidae have characteristic yellow and black stripes on the trunk. The whole family consists of paper wasps (genus Polistes), hornets (genera Vespa and Dolichovespula) and wasps (genus Vespula, see figure 2). In the USA insects belonging to genus Vespula are called yellow jackets in everyday language usage.

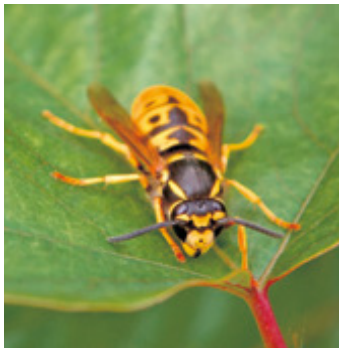


Figure 2

At first a distinction has to be drawn between Vespinae with a truncated junction around the waist and Polistes, who are characterized by a more oval shape (3). European representatives are Polistes dominulus and Polistes gallicus, mostly active in Mediterranean areas. In general they try to avoid contact to humans and therefore are of secondary importance in medicine. On the other hand the venom of Polistes is the most distantly related in the Vespidae family and even only partial cross-reactive within the genus (4). It is supposed that patients allergic to Polistes would derive benefit from a specific diagnostic and

therapeutic scheme. Up to now a standardized procedure is not widely established in Europe, but is object of further studies.

Vespa and Dolichovespula make up the two genera of hornets. Vespa crabro (see figure 3) is the most prevalent hornet in middle Europe. Anyway, compared to other Hymenoptera, they are quite rare. One medium hive consists of not more than 1000 individuals. According to color and shape they are very similar to Vespulae. Sometimes the front part can be of slightly red color. Further a distinction is possible by size: hornets are almost twice bigger than usual wasps. For generations misleading information about hornets was passed on. The old wisdom "three stings kill a human and seven a horse" cannot be verified scientifically and was never supported by reliable reports. The allergens of hornet venom as well as the injected amount are quite similar to usual wasps. Apart from that, in scientific trials the average lethal dose (ALD) of hornet venom for mice was 10 to 90 mg per kilogram bodyweight. This means compared to the venom of honeybees (ALD: 2.8 mg to 6 mg per kilogram bodyweight) a quite low toxic potential. Nevertheless, the sting of a hornet can be quite painful. Some authors suggest that Acetylcholine, beside the longer stinger, causes the stronger feeling of pain. Acetylcholine is neither a component of Apidae nor Vespulae venom.

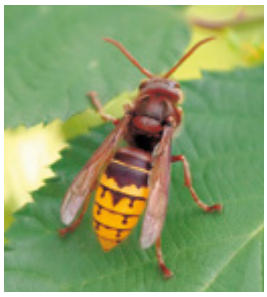


Figure 3

The most common species of the family Vespidae in middle Europe are Vespula vulgaris and Vespula germanica. It is not necessary to discriminate between the different subspecies of Vespulae, because the composition of venom is very similar within the group. A common habit of the whole genus is the preference of dark nesting places, even in buildings occupied by people. Hives of up to 7000 wasps have been reported. Vespulae are strongly attracted to human food, especially sweet food. They can be found in great numbers at outdoor events, around restaurants, leftover food or garbage cans. This circumstance, in

combination with lack of fear of humans and an aggressive character, means a high risk to get stung during outdoor activities from April to October. While other Hymenoptera tend to attack only when the critical distance to the nest is overpassed, Vespulae can sting after minor provocation. Sometimes stings occur without any obvious reason. Due to the strong attraction to sweet drinks, patients have been stung into the oropharynx and the mouth while drinking (2). In this case a local swelling, even without any systemic allergic reaction, may be life-threatening.

In all subfamilies of Vespidae only the queen and the young queens survive the winter, while the rest of the population goes down in October. Depending on climatic conditions in springtime, new hives are founded not before March. Therefore a high population of Vespidae cannot be expected before April. This circumstance can give a hint to the culprit insect, if the anamnesis is not sure and performed tests do not lead to a clear result.

2.3) Formicidae

Stings of Formicidae, who are regarded as a family of Hymenoptera, are of minor medical importance in Europe. Some cases of allergic reactions to ant venoms have been reported (5), almost all caused by Formicidae rufa.

In Northern and Central America the red fire ant (Pogonomyrmex) is mainly responsible for allergic sting reactions (3). These ants migrated from Mexico and are now endemic in wide areas of the US. Pogonomyrmex is very aggressive, especially when the nest is disturbed. Multiple stings and sterile pseudopustules, which develop at the site of the sting, are typical for a reaction to fire ant venom.

3) Hymenoptera Venom Allergens

To understand the underlying pathological mechanism of allergic reactions to Hymenoptera stings, it is inevitable to know about the constitution of venoms of the different subspecies. Overlapping amino acid sequences of allergens, causing cross reactivity and double positive in-vivo and in-vitro tests, play an important role in the correct diagnosis and subsequently in the selection of the appropriate venom for the immunotherapy.

All known insect venom allergens are glycoproteins, mainly with a molecular weight between 10 to 50 kDa, consisting of 100 to 400 amino acid residues. A distinction has to be drawn between major and minor allergens. If more than 50 % of allergic patients react to an specific allergen, it is designated as major, otherwise as minor. In table 1 the major allergens in context with the transmitting insect are listed, retrieved from the database www.allergen.org.

3.1) Venom Dose

After production in the acid glands the poison is stored in the venom sac, from where it is squeezed during the sting. The venom dose per sting varies not only from species to species, but also within the same species of Hymenoptera. In the subfamily of Vespinae the released amount per sting comes to 1.7 up to 5 μg of protein. Paper wasps inject about 4.2 to 17 μg per sting. The delivered amount of venom during a bee sting is about 10-fold higher, ranging from 52 up to 66 μg of protein (6). The complete capacity of the venom sac is delivered after 1 minute, already 90 % after 20 seconds (7). Vespidae are capable of repeated stings, which explains the lower and fluctuating venom dose. The severity of an allergic reaction to both species is mainly independent of the venom dose.

3.2) Antigenic Cross-reactivity

Multiple sensitization appears as a complicating factor in correct diagnosis of Hymenoptera venom allergy. A double positive result in a skin test, ImmunoCAP® or RAST can be caused by exposure to different Hymenoptera allergens and subsequent increased production of different venom-specific IgE, what means true double sensitization. On the other hand cross-reactivity, caused by cross-reactive carbohydrate determinants (CCDs), feigns the same diagnostic picture and leads to false-positive results. The main cause for cross-reactivity is based on CCDs: The structural basis are asparagine-linked carbohydrate moieties of multiple plant and insect glycoproteins. Mueller UR et al came to the result that double positivity is observed in up to 59 % of subjects, depending on the lab method used (8). All subjects had a convincing history of allergic reaction to only bee or wasp venom. To reduce costs and the risk of choosing the wrong therapeutic venom it is crucial to analyze positive responses critically and in context with the patient's history.

The Apidae and Vespidae group each have unique as well as homologous venom proteins. Cross-reactivity is very common within the subfamily of Vespidae, where the sequence identity of allergens ranges from 40 % up to 99 % (9). From the bee venom proteins hyaluronidase may causes cross-reactivity with Vespid hyaluronidase (9). Sequence identity of these allergens is about 50 % between the superfamilies. However, this cross-reactivity is mainly based on CCDs and less often because of shared peptide epitopes (10).

Generally, cross-reactivity of B-cell epitopes is widely detected in proteins of more than 90 % sequence identity, but rarely in proteins of less than 50 % identity (9).

3.3) Venom of Paper Wasps

The allergens of paper wasp venom are quite unique among the family of Vespidae and significant differences occur even within the genus. The limited cross-reactivity between paper wasp venom and the venom of other species of Vespidae is based on different epitopes on the allergen molecules.

Polistes dominulus and Polistes gallicus originally come from Europe, while Polistes annularis, Polistes fuscatus and Polistes exclamans are typical of North America. Specific testing and therapy with a special Polistes mix, consisting of Polistes annularis, Polistes fuscatus, Polistes metricus and Polistes exclamans, is common clinical practice in the United States. The venom composition of European Polistes is object of current studies, not least because Polistes dominulus is already endemic in North-eastern America.

As in the whole group of Vespidae, major allergens of Polistes' venom are phospholipase, hyaluronidase and antigen 5, a protein of unknown biological function. A fourth allergen, serine-protease, was detected in Polistes dominulus and Polistes exclamans. The sequences are openly available on the internet at www.uniprot.org.

Severino M et al (11) found out that the cross-reactivity between the European Polistes gallicus and Polistes dominulus is quite high, but only partial between European and American species. According to Pantera B et al (4) the sequence identity of antigen 5 is 98 % within European paper wasps, and decreases to 85 % in comparison to American paper wasps. Up to now not all details and differences between Polistes venom of American and European species have been investigated. Additional differences in the tertial structure of allergens are suspected (4). In an Italian clinical trial (11), venom of European Polistes was used for testing and treatment of 130 patients. The result was increased sensitivity of testing and improved efficiency of therapy.

Pantera B et al (4) suggest a new Polistes mix for diagnosis and therapy, including venom from European Polistes. Also in America,

where Polistes dominulus is widely spread, patients could benefit from a new composition of venoms.

Table 1

Species	Allergen	Biochemical name
Apis mellifera	Api m 1	Phospholipase A2
	Api m 2	Hyaluronidase
	Api m 5	Dipeptidylpeptidase 4
Bombus	Bom p 1	Phospholipase A2
	Bom p 4	Protease
Vespula vulgaris	Ves v 1	Phospholipase A1B
	Ves v 2	Hyaluronidase
	Ves v 5	Antigen 5
Polistes annularis	Pol a 1	Phospholipase A1B
	Pol a 2	Hyaluronidase
	Pol a 5	Antigen 5
Polistes gallicus	Pol g 1	Phospholipase A1
	Pol g 5	Antigen 5
		Hyaluronidase
		Protease
Dolichovespula maculata	Dol m 1	Phospholipase A1B
	Dol m 2	Hyaluronidase
	Dol m 5	Antigen 5
Vespa crabro	Vesp c 1	Phospholipase A1B
	Vesp c 5	Antigen 5

4) Sting Reactions and Pathogenesis

4.1) Normal Local Reaction

The majority of people reacts to injection of Hymenoptera venom by a sting with typical symptoms of inflammation: *rubor, dolor, calor, tumor*. If the sting occurs on a sensitive area e.g. fingers or around the eye, even *functio laesa* can be considered as a standard symptom. These alterations, confined to the sting site, are based on irritative and toxic effects of the venom. Usually this local condition regresses within hours and only a small inflammatory spot remains visible for a few days. To reduce the discomfort applying ice is advisable, but no further investigation or treatment is necessary.

Even in the non-allergic population stings into the mouth close to the larynx may lead to life-threatening dyspnea caused by extensive local swelling.

4.2) Large Local Reaction

To fulfill the definition of a large local reaction, the visible skin alteration (swelling, redness) has to exceed 10 cm in diameter and persist more than 24 hours. Patients may suffer from intensive pain and discomfort, especially when big joints or the face are involved. Persistence for several days or weeks is possible, sometimes accompanied by systemic inflammatory symptoms like headache, swollen lymph nodes or fever.

At present, the underlying pathomechanism is not entirely clear. Different studies found out that up to 90 % of patients with a large local reaction in history had a positive result in skin testing (12). These findings suggest an IgE mediated pathogenesis, albeit specific IgE was not found in all subjects. Other authors presume cell-mediated allergic reaction, at least as a cofactor.

However, patients with large local reactions have a slightly increased risk to develop systemic anaphylactic reactions after a sting in the future (13,14).

4.3) Systemic Toxic Reactions

Systemic toxic reactions appear after several stings. Usually more than 50 stings are necessary to provoke toxic symptoms. The time between injection of venom by stings and onset of symptoms is from hours to days (15). Like toxicity caused by any other venom the severity depends on the dose. More than 200 stings may cause fatal reaction in healthy adults. Therefore systemic toxic reactions are rare and occur mostly in especially exposed populations like beekeepers, glasshouse workers, etc. Symptoms range from hepatic dysfunction, myocardial damage and rhabdomyolysis to intravascular haemolysis, coagulation disorder and acute renal failure (15). Phospholipase and Hyaluronidase, which are part of bee and wasp venom, are supposed to be responsible for toxic reactions.

4.4) Systemic Anaphylactic Reactions

Anaphylactic reactions to Hymenoptera venom vary substantially in their clinical manifestation. Disorders range from skin to gastrointestinal, pulmonary or cardiovascular symptoms, in worst case leading to life-threatening apnea and cardiac arrest. Usually anaphylactic symptoms appear within 30 minutes after the sting (16), and complete recovery normally is reached after a few hours. Standardized classification of the clinical presentation of anaphylaxis helps to plan adequate diagnosis and treatment. Two widely established classifications designed by Mueller (table 2) and Ring & Messmer (table 3) are shown below.

Table 2

Grade 1	Generalized urticaria, itching, malaise and anxiety
Grade 2	Any of the above mentioned symptoms plus two or more of the following: angioedema, chest constriction, nausea, vomiting, diarrhea, abdominal pain, dizziness
Grade 3	Any of the above mentioned symptoms plus two or more of the following: dyspnea, wheezing, stridor, dysarthria, hoarseness, weakness, confusion, feeling of impending disaster
Grade 4	Any of the above mentioned symptoms plus two or more of the following: fall in blood pressure, collapse, loss of consciousness, incontinence, cyanosis

Table 3

Grade 1	Generalized skin symptoms including flush, generalized urticaria, angioedema
Grade 2	Mild to moderate pulmonary, cardiovascular, gastrointestinal symptoms
Grade 3	Anaphylactic shock with loss of consciousness
Grade 4	Apnea, cardiac arrest

It is generally accepted that anaphylactic transmission is mostly caused by IgE-mediated stimulation of prior sensitized mast cells, inducing degranulation and release of histamine, prostaglandins and leucotriens. Specific IgE and positive skin test results are found in the majority of patients who underwent systemic anaphylaxis after Hymenoptera stings.

Rare reported cases of non-IgE mediated systemic anaphylactic reactions to Hymenoptera venom (17,18) suggest supplementary pathways of mast cell stimulation. Some authors assume that short term IgG antibodies in association with factors of the complement

system may play a role in the pathogenesis of anaphylaxis (12). Anyway, angioedema is a common symptom of Hymenoptera venom allergy and it is a fact that this alteration can also occur via activation of the complement system. Thus release of complement factors could be a possible reason for anaphylactic reactions in IgE-negative patients.
Furthermore radio contrast media–like stimulation of mast cells based on activated anaphylatoxins of the complement system is supposed to be an explanation for non-IgE mediated anaphylaxis.
It is important to differentiate between systemic symptoms depending on immunological mechanisms and vasovagal reactions after the sting. Beside typical anaphylactic symptoms like urticaria, angioedema and stridor, plenty of subjective symptoms can occur: itching, paraesthesia, headache, nausea, dizziness, fear etc. If a patient shows no symptoms that clearly proof an anaphylactic reaction and all tests including blood samples remain negative after repeated testing, a panic reaction should always be taken into consideration.

4.5) Atypical Reactions

There are some case reports about atypical reactions to Hymenoptera venom, most of them associated with neurological disorders or serum sickness like symptoms. The relation between sting and the respective medical condition is often doubtful, and immunological mechanisms are unclear. Due to only few reported cases for each disease suspected to be related to a Hymenoptera sting, systematic studies with high validity do not exist.

5) Epidemiology of Pathological Sting Reactions

The prevalence of Hymenoptera stings in general is very high, depending on location (rural, urban), outdoor activities and profession. With a prevalence of up to 19 % the large local reaction is the most common pathological sting reaction (15).

Despite the high number of sensitizations detected by skin testing and blood samples, the prevalence of systemic sting reactions is low. In different studies, 0.5 to 7.5 % of the general population suffers from systemic reactions to Hymenoptera venom (3,19). However, in practice it is difficult to distinguish real anaphylactic reactions from systemic symptoms caused by vasovagal disorder.

The registered number of fatal reactions every year in Germany varies from 10 to 20 (15). The incidence of fatalities is stated in different studies from 0.03 up to 0.48 per 1,000,000 inhabitants (19), of course depending on the climatic zone.

The true number of victims may be underrated. However, panic is not indicated. One author found venom specific serum IgE in 23 % of victims who died unexpected outdoors during the time of Hymenoptera activity (20). To put this result into perspective: approximately 25 % of the normal population has increased specific IgE-levels in serum without being allergic to any kind of Hymenoptera (15).

On the other hand up to 85 % of people dying from Hymenoptera stings have no history of previous anaphylactic symptoms (19,21). Therefore one has to assume that by far not all deaths caused by allergic reaction to Hymenoptera venom are detected.

Adequate immunotherapy and emergency sets could prevent most sting-related deaths. An alarmingly high number - 90 % of allergic patients – are not treated sufficiently or equipped with emergency medication.

6) Diagnosis

Diagnosis of Hymenoptera venom allergy is not always straightforward. Hymenoptera sting many people every year, nevertheless only a small number experiences systemic symptoms. The aim is to find out whether a patient requires treatment with emergency medication or immunotherapy with bee or wasp venom. Therefore information is needed on:

- The severity of the clinical symptoms
- The pathological mechanism
- The species of the culprit insect

In case the sting reaction was not caused by bees or wasps, a standardized test protocol does not exist. Usually, due to the close relationship between the different species within one family, it is only necessary to distinguish between stings caused by Apidae or Vespidae. Today several modern in vitro test methods are available but not always efficient, so any decision has to be done step by step. The following scheme gives some direction for the correct management of patients with a history of a systemic sting reaction.

6.1) Taking the History

Taking an exact history can give a clue to the culprit insect. Information is needed on:

- Location at the time of the sting
- Activity at the time of the sting
- Insect suspected by the patient

Further should be taken into account that:

- Wasps tend to be more active in summer and autumn, while bees appear already in springtime and even on warm winter days.
- Only wasps are capable of multiple stings.

– Bees usually lose their stinger after one sting, but they do not die immediately. A stinger that remains in the skin is an indicator for a bee sting, even if the insect itself was not identified.

The worldwide accepted classifications of Mueller and Ring & Messmer allow a standardized grading of the subjective symptoms reported by the patient. The time between the incident and the onset of symptoms is helpful to rule out unusual sting reactions. If a patient suffered numerous stings, the possibility of a systemic toxic reaction has to be considered.

Finally the patient should be asked for physical and psychological risk factors, previous anaphylactic reactions, exposure to bees and wasps as well as associated insects. Furthermore, the intake of special medication (β-blockers, ACE-inhibitors) must be considered.

6.2) Skin Tests

If venom immunotherapy might be indicated after taking the personal history, a skin test should be performed to control the presence or absence of mast cell bound venom-specific IgE antibodies. The principle is to provoke a skin reaction in a patient by adding a minor amount of allergen. The most accepted method is the intradermal endpoint titration. Sometimes, a prick test is recommended before the intradermal test, but the diagnostic benefit is in doubt and regarding the meaningfulness it does not reach the intradermal testing method. Some allergists take into account that the risk of inducing anaphylactic reactions is lower in prick testing than in intradermal testing. Anyway, the overall risk of causing severe adverse reactions is very small in skin testing (22,23).

Skin tests to Hymenoptera venom are usually performed on the forearm. To avoid iatrogenic infections and false positive results it is important to clean the tested skin area in advance. For correct interpretation venom should not be administered closer than 3 cm to

the last concentration. As in other skin tests, a positive control with histamine solution and a negative control with sodium chloride solution is necessary.

It is widely recommended to administer venom stepwise with waiting time between each application. If the first concentration does not provoke a clear reaction within 15-20 minutes, a 10-fold increased dose is administered, up to the maximum for each test. When the patient shows a significant positive result in form of a wheal and flare reaction, the endpoint titration can be stopped. The advantage of this procedure is that the patient receives the smallest necessary amount of venom.

On the other hand it takes a long time if the patient does not react at all or not until the maximum dose is reached. Depending on the first concentration the whole test takes up to 2 hours. Our experience with time-saving simultaneous intradermal testing is described detailed at the end of this publication.

Intradermal testing starts with a concentration from 0.00001 to 0.01 μg/ml, up to the maximum of 1 μg/ml. The sensitivity of intradermal testing after administration of 1 μg/ml is more than 90 %, but a positive response to this maximum dose can also be caused by enzyme activity (24). Because of this reason the administration of higher doses has no diagnostic benefit and leads only to more false positive results. A positive result is determined as a wheal of more than 5 mm in diameter. Per test concentration, 0.02 ml are injected with a thin needle into the dermis. The way of application sometimes causes short vasovagal episodes that may copy allergic symptoms. Real anaphylactic reactions occur very rarely during intradermal testing (23).

Generally, for the skin prick test higher doses are necessary (0.1-1-10-100 μg/ml). Some allergists even set the endpoint at 300 μg/ml. A small drop of each allergen extract is applied to the forearm, and then the skin is pricked with a sharp device. A positive result is again determined as a wheal (> 3 mm) and flare reaction. Performing a prick test is uncomplicated, cheap and less painful for the patient, especially

for children. Up to 100 μg/ml the specificity is high but decreases with increased doses. Compared to intradermal testing the sensitivity of a prick test is lower. Jeep et al showed that the intradermal test is slightly more specific at the same level of sensitivity, especially at higher concentrations (24). For this reason an additional intradermal test has to be performed if prick testing does not produce a clear result, which means further discomfort for the patient.

The reproducibility of both tests is relatively poor. A study of Graif Y et al demonstrated that after 4 weeks or more the intradermal test was only in 66 % of patients reproducible (25). A common problem in skin testing is the refractory period after a sting. Cautious interpretation of negative results obtained in the first few weeks after a sting reaction is advisable. Goldberg A and Cohen R found out that more than one fifth of sensitive patients could not be identified in the first week after a documented sting reaction (26). Testing within two weeks after an assumed anaphylactic reaction is not recommended. Additionally, testing should be repeated after one to two months in case of an initial negative test.

Both methods of skin testing pose a minimal risk of systemic reaction to the administered venom. Therefore prepared emergency medication is recommended, as well as further monitoring after administration of the last dose.

6.2.1) Interpretation of Skin Tests

It is important to read skin tests not earlier than 15-20 minutes after administration of the final dose. In rare cases of non-anaphylactic sting reactions the late result 48-72 h after skin testing can be helpful for diagnosis and further treatment (15).

It is essential to compare wheals caused by administration of venom with the positive and negative control. The histamine solution shows the general ability of the skin to anaphylactic reaction. The sodium chloride

solution helps to rule out false positive results based on urticaria factitia. For documentation the size of wheals should be drawn or noted in mm, also of the positive and negative control. Prick tests and intradermal tests are considered to be positive if a wheal of > 3 and 5 mm in diameter with erythema is observed, respectively.

One should take into account that a prediction of further sting reactions is not possible by any test method. Up to 84 % of patients with a positive skin test do not react to a further field sting caused by the same insect (3,27). This data imply the fact that most patients can not reliable distinguish between bees, wasps and hornets. Therefore a field sting does not reach the validity of a controlled sting provocation test under clinical circumstances.

In both test methods a positive result, especially after administration of the prevailing maximum venom dose, can be caused by toxic reaction. About 25 % of tested subjects respond to skin tests without clinical relevance, among children every second (15). Under atopic disposition false positive results are even more likely. Regarding the fact that only about 2 % of the population suffer from clinical relevant symptoms after Hymenoptera stings, the specificity is quite low. Therefore a skin test represents just one part in the diagnosis of Hymenoptera venom allergy and should never be performed without relevant history of a sting reaction; this is also valid for all other available diagnostic tools. The decision for a venom immunotherapy should be made after confirming the personal history by identification of an IgE-dependent mechanism.

6.2.1.1) Negative Test Results after Systemic Reaction

Although the sensitivity of Hymenoptera venom skin tests is very high, there are patients with a positive sting history who do not respond even after repeated testing and exclusion of interfering factors like medication, eczema or insufficient application (17,18). Current guidelines recommend that venom immunotherapy should be started

only if the presence of specific IgE is proven with skin tests or other diagnostic methods. But there is no commonly accepted opinion how to manage patients with a convincing systemical sting reaction and a negative allergy workup.

Some authors suggest sting challenges in a specialized center, after unspecific reactions have been excluded (17), while others refuse performing sting challenges on patients without previous venom immunotherapy.

In some patients with inconclusive diagnostic results it is possible to detect specific IgE with more sensitive lab tests. A quite new method is the basophil activation test. One study detected in 75 % of patients with a Grade 2-3 reaction in personal history and negative conventional allergy workup a positive CD 63 basophil response (28).

Since Hymenoptera venom allergy is a potentially fatal condition, negative results in combination with prior systemic reactions have to be analyzed in any case.

6.2.2) Contraindications for Skin Tests

Serious incidents after skin tests with Hymenoptera venom are rare and do not occur more frequently than in other commonly performed skin tests. Nevertheless under the circumstance of

- Pathology in the tested skin area , e.g.
    - Eczema
    - Sunburn
- Acute infection diseases

a prick or intradermal test should not be performed. Antiallergic drugs and some psychopharmaceutical agents may cause false negative results. The necessary drug-free intervals are listed in table 4.

Table 4

Medication	Drug-free interval
Oral antihistamines	3 to 10 days
Prednisolone (more than 10mg/d)	7 days
Topical glucocorticoids	7 days
Psychopharmaceutical agents	5 days

6.3) Venom-specific IgE

It has been recognized that in few patients venom-specific IgE are present, which are not detectable by skin testing. Therefore the next step in further evaluation procedure is to perform an Immunoassay (e.g. ImmunoCAP®), a method derived from the **R**adio **A**llergo **S**orbent **T**est. A negative test should be repeated after one to two months.

Immunoassays and related tests are based on the principle of antigen-antibody binding (see figure 4). The positive correlation between skin test and specific IgE in serum indicates a high validity of both methods (29). The sensitivity of Immunoassays is up to 100 % (8). Additional measurement of total serum IgE-level is an obligation for correct interpretation. The notation of RAST-classes is obsolete and not satisfyingly exact (due to a wide range within one class), but still common practice.

It has to be considered that specific IgE are low or even not detectable within a few days after the sting. This initial rapid decline may help to identify the culprit insect in difficult cases. The maximum of allergen-specific IgE is reached within weeks after the sting, before antibody-levels decline slowly with a large individual variation.

CAVE: Depending on the total IgE level, 23.1 to 66.7 % of the population has increased venom-specific IgE without clinical consequences (30). This is why an IgE test should never be performed

as a screening method, but only after a suspect sting reaction. False positive results are even more likely in combination with high total serum IgE. One the other hand false negative results may occur in patients with low total IgE levels.

6.4) Baseline Serum Tryptase

Tryptase is a mediator substance of mast cells and is stored intracellular. Mast cell burden can be estimated by measuring the baseline serum tryptase concentration. It is thought to reflect the individual mast cell activity. As a predictor of potential fatal anaphylactic reactions, the determination of baseline serum tryptase is compulsory in patients who underwent severe anaphylactic episodes (31). The German Association for Allergy and Clinical Immunology (DGAI) defines the cut-off for elevated baseline serum tryptase as a sign for increased risk at 10 μg/l (15).

6.5) Basophil Activation Test

The basophil activation test, based on a flow cytometric assay, is a quite new but widely validated method to measure the response of basophils after allergen exposure. A commonly used marker to determine activation is CD63, which is demonstrated on the external membrane of basophils after degranulation.

At present performing this test is only possible in specialized laboratories and it will not become a standard procedure in the near future. An unsolved problem is the time factor: Tested blood samples should not be stored longer than 4 hours because of negative effects on the results. Anyway, the present data are very promising. Treatment with antihistamine drugs has no influence on the outcome of the basophil activation test – an advantage over skin testing.

6.6) Sting Challenge Test

Clinical sting challenge tests with standardized insects are the gold standard to reveal the individual responsiveness to a certain kind of venom. According to current guidelines a sting challenge test should only be performed in patients on maintenance venom dose to control the efficacy of immunotherapy (3).

Some authors see in the sting challenge test an appropriate instrument to detect patients without need for further treatment. The leading thought in this approach is that only about 20 to 40 % of subjects with a history of systemic sting reaction will react when they get stung again by the same insect (32,33). Compared to the sensitivity of skin testing (> 90 %) this means a quite low risk for further systemic reactions. According to this diagnostic scheme the number of patients who require venom immunotherapy would decrease markedly. Unfortunately a negative sting challenge test does not fully exclude further systemic reactions to the tested venom. Moreover, it should be taken into account that a provocation test without previous venom immunotherapy represents a risk for severe anaphylaxis.

7) Predictors for Severe Reactions

Not all parameters that were suspected to predict more severe systemic reactions after exposure to Hymenoptera venom turned out to be true. Mosbech H found out that the severity of a sting reaction does not correlate with concentration of total serum IgE, atopic disposition, number of stings during life and positive skin prick test to inhalative allergens (29).

Rueff F et al came to the result that the probability of further severe events is significantly increased after former episodes of grade 3 or 4 anaphylaxis according to Ring & Messmer (31). An entire diagnostic workup in a specialized clinic is strongly recommended, including several lab tests (see chapter 6).

Independent studies demonstrated that patients with elevated baseline serum tryptase show more severe allergic symptoms after field stings than patients with normal concentrations (34,35). Therefore, according to the guidelines of EAACI (3) the baseline serum tryptase should be determined in all patients who underwent severe reactions.

Beta blockers, ACE-inhibitors and non-steroidal anti-inflammatory drugs are suspected to play a role in the pathological progress of anaphylaxis (3,31).

While systemic reactions are mostly mild in children, elderly patients – especially in combination with cardiovascular diseases – show more often severe symptoms (31). Also asthma can act as a cofactor for serious bronchoconstriction.

Stings that occur shortly after a previous sting may determine more severe systemic reactions. This fact has to be considered if the patient belongs to an intensively exposed group, like gardeners, beekeepers, farmers, bakers, food handlers, greenhouse workers etc. An interesting finding is that being stung very frequently appears to induce natural tolerance, comparable to venom immunotherapy. Bousquet J et al found

out that none of the beekeepers who got stung more than 200 times per year developed an anaphylactic reaction (36).

Based on data from clinical sting challenges before treatment and observations during venom immunotherapy the general opinion is that bee venom allergy tends to result in more severe systemic symptoms than allergy to vespid venom. In a recent observational multicenter study with 962 patients, Rueff F et al came to the result that this seems to be untrue for field stings. Potentially life-threatening symptoms were significantly more common in the vespid venom allergic group (31). The findings of Antonicelli et al (37), who presumed a three times higher risk for life-threatening conditions after stings by Vespa crabro, were not confirmed by other authors.

8) Patients, Material and Methods

This retrospective study was carried out at the Division of Environmental Dermatology and Venerology of the Medical University Graz, where simultaneous intradermal skin testing with Hymenoptera venom was introduced in 2002.

Medical records of 484 patients with a history of at least one anaphylactic reaction after a Hymenoptera sting were evaluated. All patients were tested between 2002 and 2010. Physicians were requested to perform simultaneous intradermal skin tests under following directive:

1. Disinfection on the forearm.
2. For safety reasons, an intravenous line was placed into a vein of the opposite arm.
3. Intradermal injection of Alk wässerig® SQ in the concentrations of 0.00001, 0.0001, 0.001, 0.01, 0.1 and 1μg/ml. Of each concentration 0.02 ml were administered with a thin needle into the dermis. Different venom dosages were injected consecutively with different syringes, but without waiting time between the injections.
4. Twenty minutes after the administration of venom the size of wheals and erythema was documented.

All subjects had a convincing history of anaphylactic reaction after a Hymenoptera sting. They were not recruited by age, gender and severity of anaphylaxis or other risk factors.

244 male and 240 female were enrolled, the age at the time of testing ranged from 8 to 80 years (median 40; 25, 75 % percentiles: 28, 54).

The severity of anaphylactic reaction was determined according to the classification of Ring & Messmer. On average, the grade of anaphylaxis in the collective was 2.3. In table 5 the observed frequency of each grade is listed.

Table 5: Grade 0: large local reaction

Grade	n (484)	Frequency (%)
0	6	1.2
1	30	6.2
2	283	58.5
3	158	32.6
4	7	1.5

9) Results

More severe anaphylactic reaction after the initial field sting was associated with male gender. Only 69 (28.8 %) of 240 female patients, but 96 (39.3 %) of 244 male patients reported grade 3 or 4 symptoms (see figure 5 and 6).

Figure 5: Grade 0: large local reaction

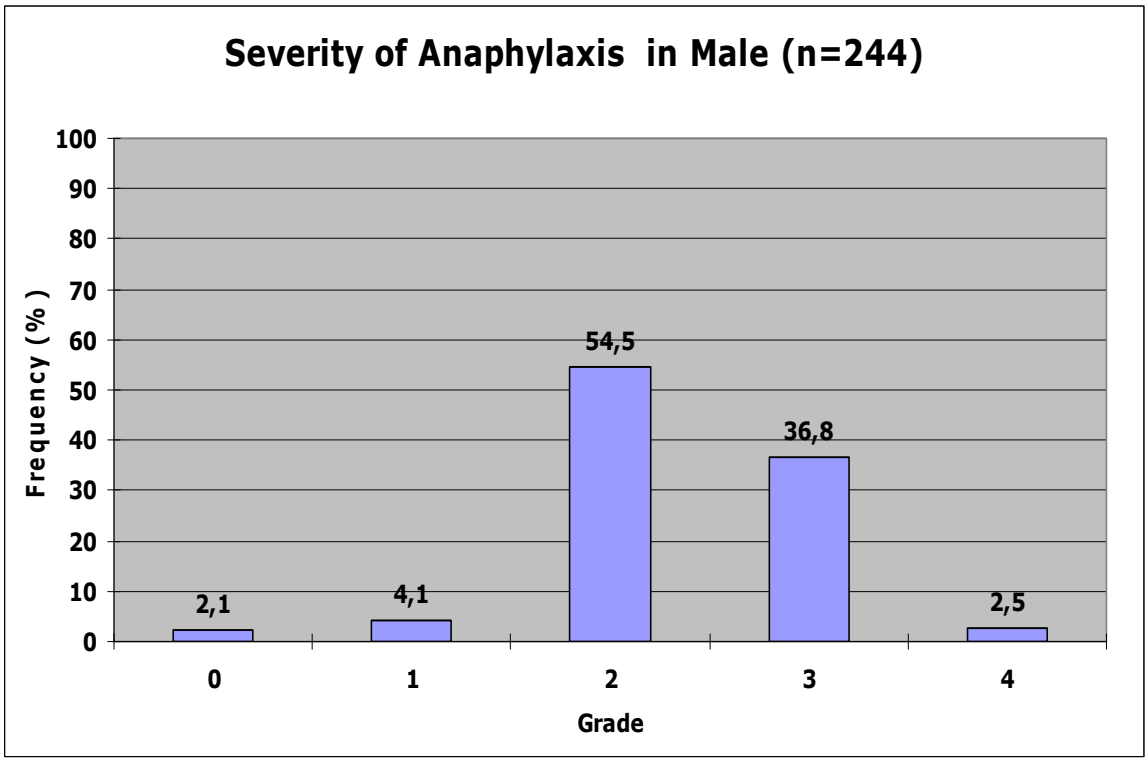
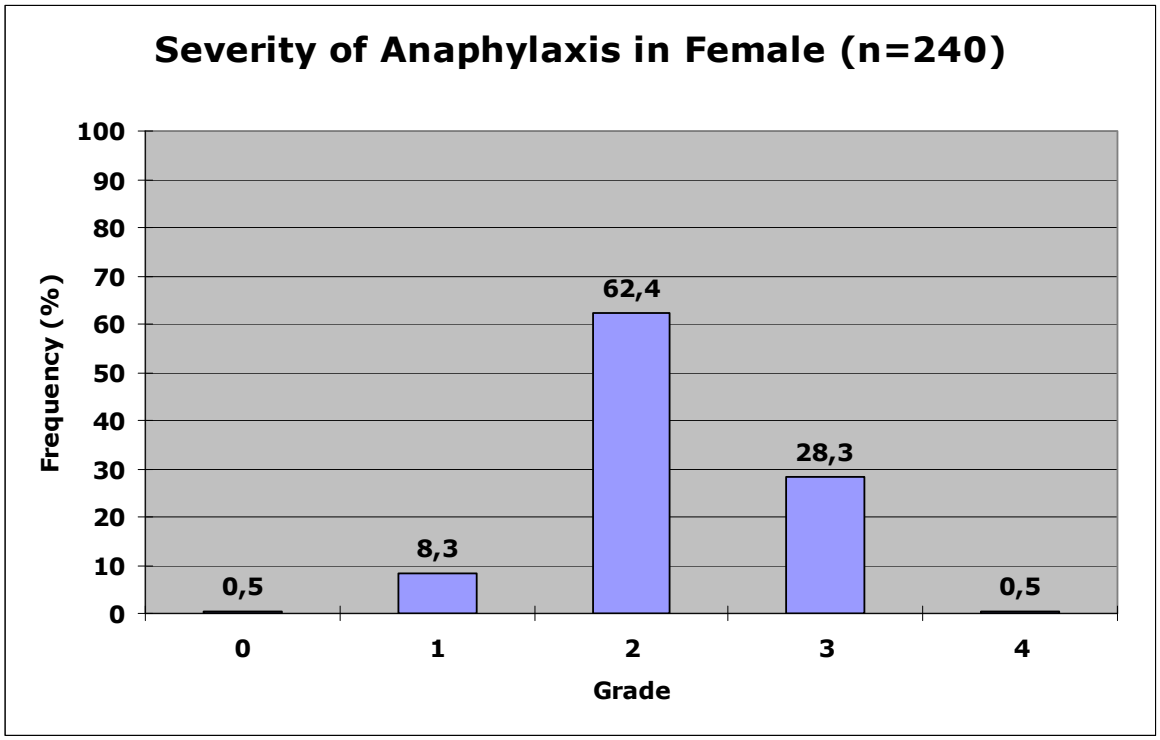
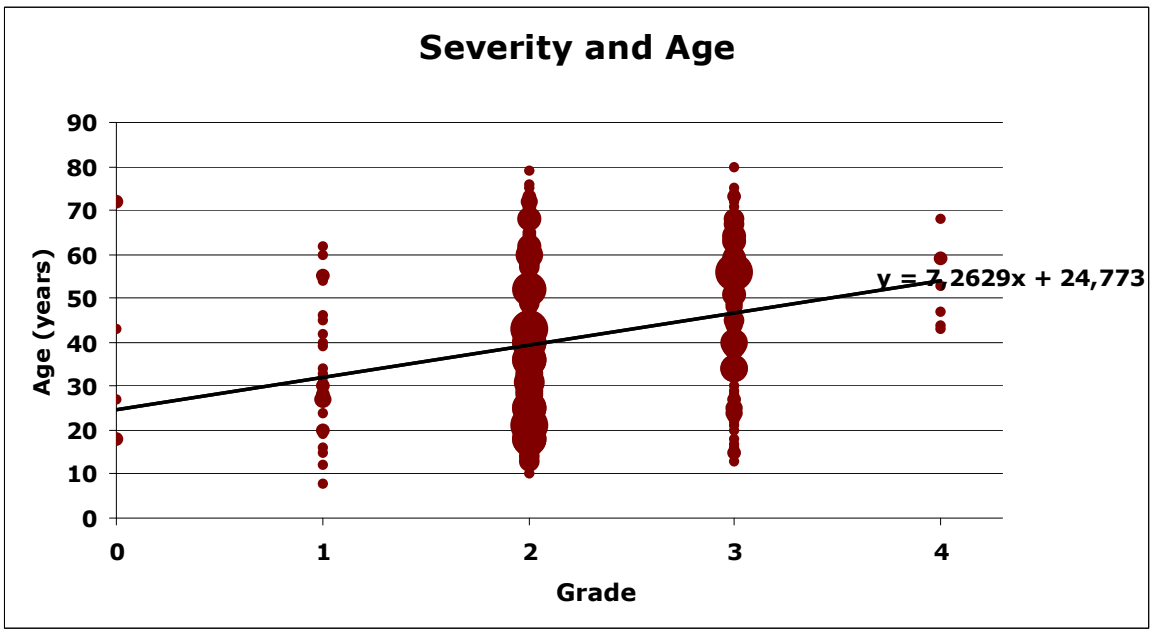


Figure 6: Grade 0: large local reaction



Regardless of the gender, age is a generally accepted risk factor for severe anaphylaxis. Our data showed coherence between age and severity of anaphylaxis after the initial field sting (see figure 7). Elderly patients tend to more severe anaphylactic reactions ($p < 0.0001$).

Figure 7: Grade 0: large local reaction

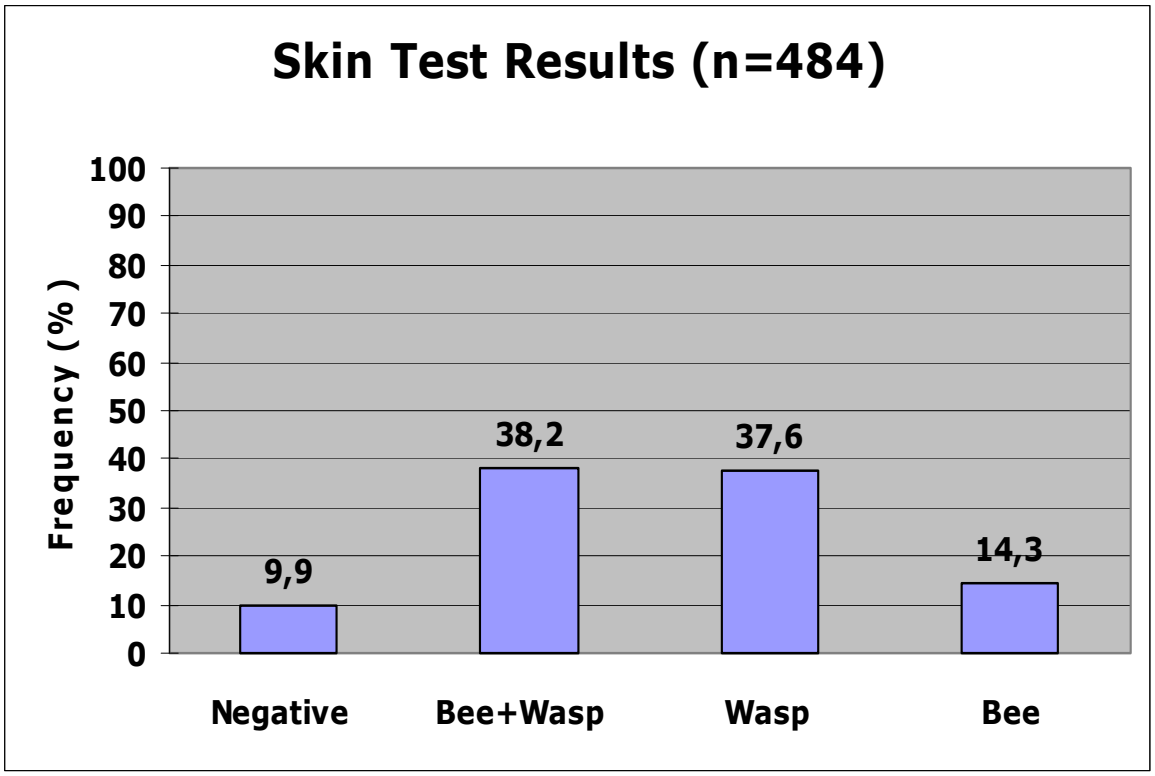


9.1) Skin Test Results

48 subjects did not respond to any of the tested venom concentrations. 185 subjects responded to bee and wasp venom and were considered as double positive. 69 patients showed only a positive reaction to bee venom. 182 patients were negative for bee venom, but had a positive response to wasp venom (see figure 8).

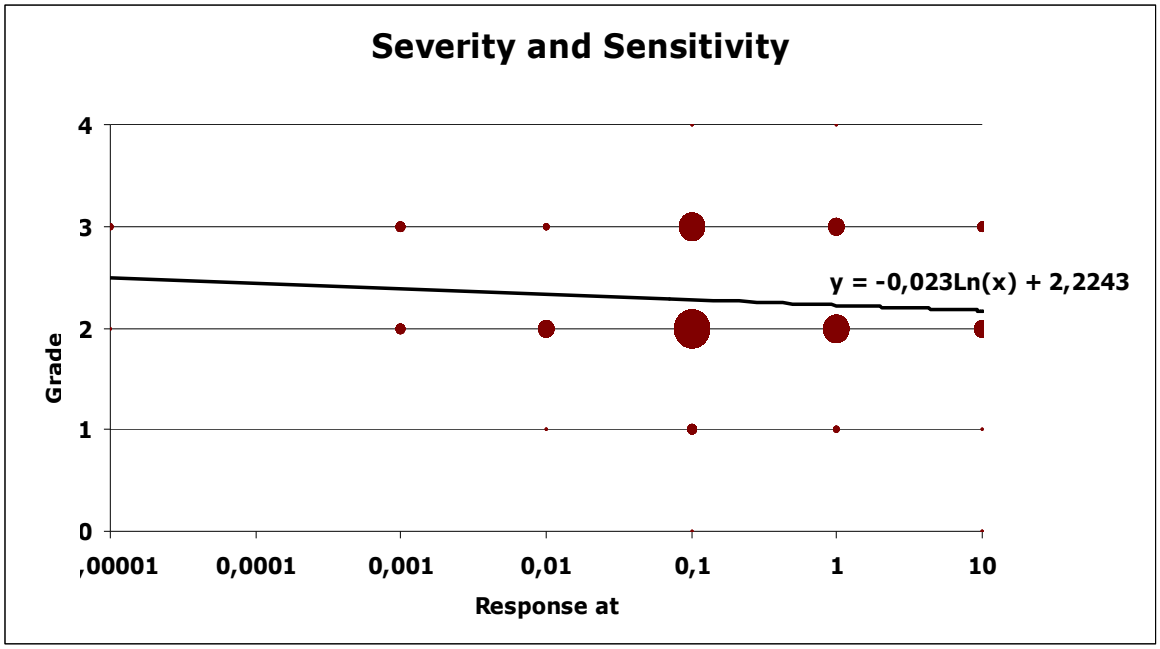
The prevalence of a positive test to at least one venom was 90.1 %.

Figure 8



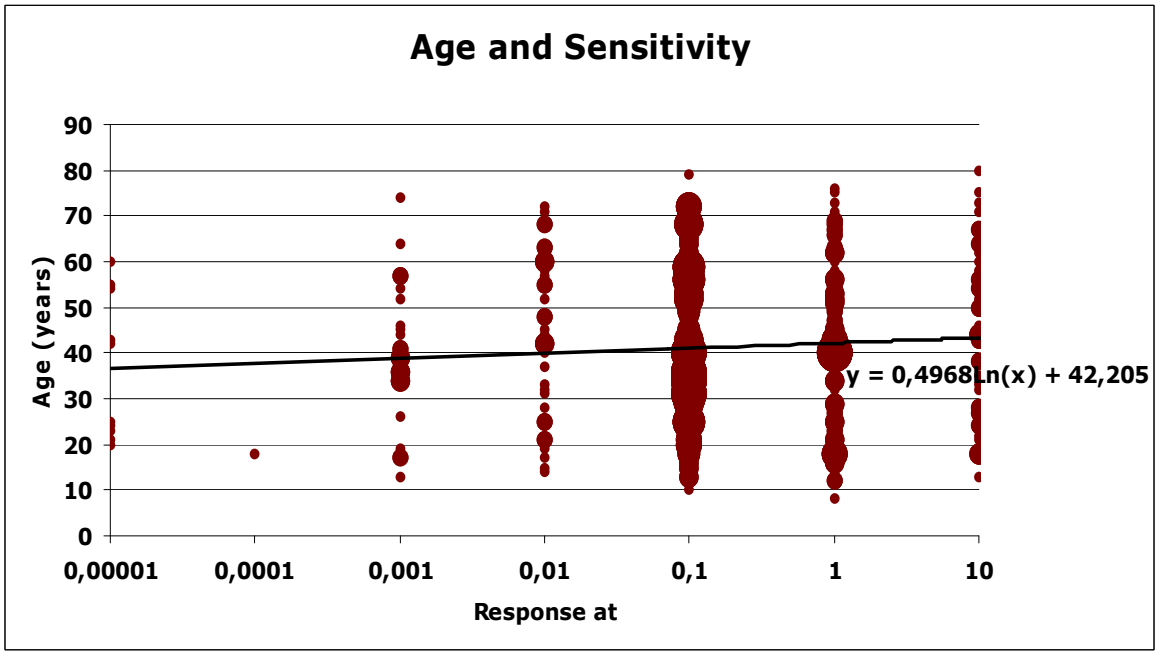
The severity of the initial field sting reaction correlated with the sensitivity in intradermal testing. Patients who underwent more severe anaphylactic reactions tend to respond to lower venom concentrations ($p < 0.044$). As illustrated in figure 9, this correlation is very weak (r-squared analysis 0.0063) and therefore irrelevant for clinical practice.

Figure 9: Response at 10: negative result



Another interesting finding was the relationship between age and sensitivity in intradermal testing. Elderly patients tend to decreased responsiveness. The p-value for this trend is 0.083, r-squared analysis 0.041 (see figure 10).

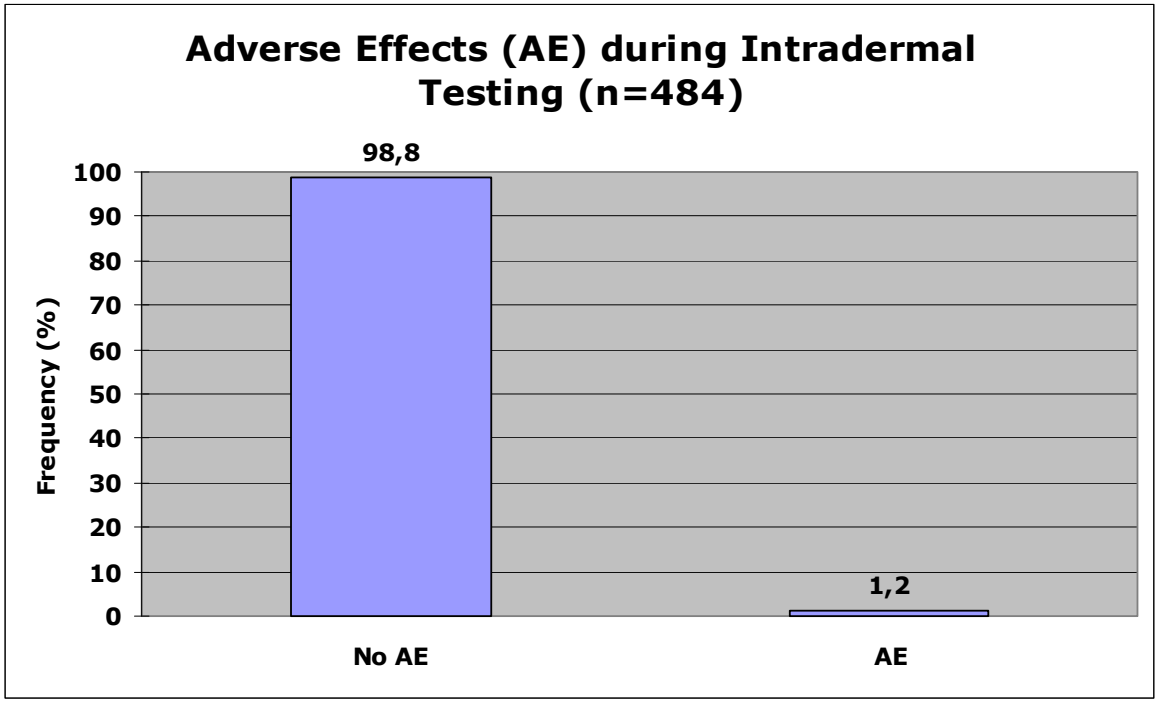
Figure 10: Response at 10: negative result



9.2) Incidences

The overwhelming majority of our subjects tolerated the simultaneous intradermal test without complains (see figure 11). 6 of 484 tested patients suffered from adverse reactions. These cases are described in detail below.

Figure 11



Two patients with a grade 2 anaphylaxis in history almost collapsed after inserting the intravenous line, but recovered immediately without treatment. Interestingly, the subsequent tests for bee and wasp venom were negative in both patients.

In one case the tested patient suffered from pre-collapse after intradermal administration of venom. Complete recovery was reached without treatment; the intradermal test was again negative for bee and wasp venom.

One 72 year old female patient with a grade 2 anaphylaxis in history after a hornet sting felt slight dizziness during testing. The result in this case was negative for bee venom, but positive at 0.1 and 1 μg/ml of wasp venom.

One male patient suffered from dyspnea, sensation of heat and dizziness after injection of venom. After the initial field sting he experienced a grade 3 anaphylaxis. He showed positive test results for bee and wasp venom at 1 μg/ml.

One 29 year old female patient suffered from wide spreading erythema on the site of the skin test, nausea and ague. No signs of tachycardia or hypotension were observed. After intravenous administration of 250 mg prednisolone and 4 mg dimetindene the patient recovered completely. Nevertheless monitoring was arranged for one night. Test results in this case were positive at 1 μg/ml of wasp venom and negative for bee venom. According to the patient's personal history, complaints during testing were comparable to the situation after the field sting (probably caused by a wasp).

10) Discussion

As mentioned earlier, the sensitivity of intradermal testing is more than 90 % at 1 μg/ml venom concentration (see chapter 6.2). We assume that our negative results (9.9 %) reflect the normal insufficiency of this procedure. Other authors described average responsiveness ranging from 63 to 76 % (23,24). Probably this discrepancy is based on different techniques in testing, for example the volume of injected solution, varying maximum concentrations and different criteria for positivity.

It is a well-known fact that wasp venom allergy is significantly more common than allergy to bee venom. This explains the different frequency of positive results to wasp and bee venom (362 versus 254, including double positive results; see figure 8).

The weak correlation between severity of anaphylactic reaction after the field sting and intradermal test results (see figure 9) is not supported by all authors. Lockey RF et al came to the result that a significant

correlation between skin test response and severity of historical sting reactions does not exist (23). However, this study considered only the wheal size of one intradermally applied test concentration, which was low concentrated (0.0001 to 0.001 μg/ml). Consistent with our results, already Mosbech H described a connection between responsiveness in skin testing and severity of sting reactions (29).

The occurrence of adverse effects during skin testing has to be viewed critically: discrimination between anaphylaxis and vasovagal symptoms is required. Many affected patients perceive the clinical situation as potentially dangerous. Under this emotionally charged circumstance vasovagal reactions are even more likely and may feign anaphylactic symptoms.

In our trial 6 patients (1.2 %) suffered from systemic symptoms during the simultaneous intradermal test. At least two of them can be considered as vasovagal: the systemic reactions occurred immediately after inserting the intravenous line, before administration of any venom. Additionally, the subsequent tests were negative for bee and wasp venom, which confirms the presumption of vasovagal symptoms. Even in a third patient who almost collapsed after the injection of venom, the skin test remained negative.

3 patients (0.6 %) suffered from systemic symptoms during testing and had a positive skin test. Two of them showed only a positive result at the maximum concentration of 1 μg/ml. Therefore also in a stepwise performed skin test the administrated amount of venom would have been the same. One elderly patient, who suffered a grade 2 anaphylaxis after a hornet sting, felt slight dizziness during testing. The result in this case was negative for bee venom, but positive at the 0.1 and 1 μg/ml concentration of wasp venom. Following the current guidelines that recommend a stepwise approach, the maximum concentration of wasp venom would not have been injected. Maybe in this case the patient would have benefited from a stepwise intradermal test. Anyway, the

systemic reaction was mild and dizziness can also occur unspecifically due to the advanced age (72 years) of this patient.

Only one characteristic anaphylactic reaction was observed during 484 simultaneous intradermal tests: a 29 year old female patient suffered from wide spreading erythema on the site of the skin test, nausea and ague. No signs of tachycardia or hypotension were observed. The skin test was positive at the 1 μg/ml concentration of wasp venom and negative for bee venom. Therefore also in a stepwise test the maximum concentration of bee and wasp venom would have been administered.

It is difficult to answer the question whether simultaneous intradermal testing causes more frequently adverse effects than the stepwise endpoint titration. Large studies investigating side effects of stepwise intradermal skin testing are rare. In a study by Lockey RF et al with 3236 patients 2 % underwent systemic symptoms during intradermal testing (23). 20 % of the systemic reactions were supposed to be based on vasovagal mediation. Administration of venom in this study was not stepwise: only one concentration between 0.0001 and 0.001 μg/ml was injected. This approach may reduce the risk of systemic reactions, but does not reach the sensitivity of higher doses. Following the simultaneous scheme (see p.27), the administered amount of venom is still low. 0.02 ml of the maximum concentration (1 μg/ml) contain 1/1000 part of venom injected during an average wasp sting, and only 1/5000 part of an average bee sting.

Compared to other invasive medical procedures, the risk of systemic reactions is slightly increased in intradermal skin testing. For instance the risk during skin prick test with aeroallergens is 0.05 %, during a venipuncture 0.5 % (38). Therefore, intradermal tests should only be performed in specialized centers and emergency medication should be immediately available.

11) Conclusion

Systemic anaphylactic reactions are a possible adverse effect during simultaneous intradermal testing, but occur very rarely. Simultaneous intradermal testing is a time-saving and safe method in the diagnosis of Hymenoptera venom allergy. Nevertheless, large prospective studies are needed to evaluate the frequency of side effects during simultaneous intradermal testing.

12) References

1. Bellmann H. Bienen, Wespen, Ameisen. Hautflügler Mitteleuropas. Kosmos Naturführer, Stuttgart 1995.
2. Moffitt JE, Golden DBK, Reisman RE, Lee R, Nicklas R, Freeman T et al. Stinging insect hypersensitivity: A Practice Parameter Update. J Allergy Clin Immunol 2004;114:869-86.
3. Bilo MB, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JNG, Birnbaum J et al. Diagnosis of Hymenoptera venom allergy. Allergy 2005;60:1339-49.
4. Pantera B, Hoffman DR, Carresi L, Cappugi G, Turillazzi S, Manao G et al. Characterization of the major allergens purified from the venom of the paper wasp Polistes gallicus. Biochim Biophys Acta 2003;1623:72-81.
5. Seebach JD, Bucher C, Anliker M, Schmid-Grendelmeier P, Wüthrich B. Ant venoms: a rare cause of allergic reactions in Switzerland. Schweiz Med Wochenschr 2000;130:1805-13.
6. Hoffmann DR, Jacobson RS. Allergens in Hymenoptera venom 12: How much protein is in a sting? Ann Allergy 1984;52:276-8.
7. Schumacher MJ, Tveten MS, Egen MB. Rate and quantity of delivery of venom from honeybee stings. J Allergy Clin Immunol 1994;93:831-5.
8. Mueller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and Vespula venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v 5. Allergy 2008;64:543-8.
9. King TP, Guralnick M. Hymenoptera allergens. Clin Allergy Immunol 2008;21:237-49.
10. Jin C, Focke M, Leonard R, Jarisch R, Altmann F, Hemmer W. Reassessing the role of hyaluronidase in yellow jacket venom allergy. J Allergy Clin Immunol 2010;1:185-90.
11. Severino MG, Campi P, Macchia D, Manfredi M, Turillazzi S, Spadolini I et al. European Polistes venom allergy. Allergy 2006;61:860-3.
12. Golden DBK, Marsh DG, Kagey-Sobotka A, Freidhoff L, Szklo M, Valentine MD et al. Epidemiology of insect venom sensitivity. JAMA 1989;262:3269-70.
13. Graft DF, Schuberth KC, Kagey-Sobotka A, Kwiterovich KA, Niv Y, Lichtenstein LM et al. A prospective study about the natural history of large local reactions after Hymenoptera stings in children. J of Pediatr 1984;104:664-8.
14. Mauriello PM, Barde SH, Georgitis JW, Reisman RE. Natural history of large local reactions from stinging insects. J Allergy Clin Immunol 1984;74:494-8.

15. Przybilla B, Rueff F, Fuchs T, Pfeiffer C, Rakoski J, Stolz W et al. Insektengiftallergie – Leitlinie der Deutschen Gesellschaft für Allergologie und klinische Immunologie (DGAI). Allergo J 2004;13:186-90.
16. Greenberger PA, Rotskoff BD, Lifschultz B. Fatal anaphylaxis: postmortem findings and associated comorbid diseases. Ann Allergy Asthma Immunol 2007;98:252-7.
17. Zidarn M, Kosnik M, Drinovec I. Anaphylaxis after Hymenoptera sting without detectable specific IgE. Acta Dermatoven APA 2007;16:31-3.
18. Golden DBK, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Insect sting allergy with negative venom skin test responses. J Allergy Clin Immunol 2001;107:781-2.
19. Bilo MB, Bonifazi F. The natural history and epidemiology of insect venom allergy: clinical implications. Clin Exp Allergy 2009;39:1467-76.
20. Schwartz HJ, Squillace DL, Sher TH, Teigland JD, Yunginger JW. Studies in stinging insect hypersensitivity: postmortem demonstration of antivenom IgE antibody in possible sting-related sudden death. Am J Clin Pathol 1986;85:607-10.
21. Mosbech H. Death caused by wasp and bee stings in Denmark 1960-1980. Allergy 1983;38:195-200.
22. Liccardi G, D'Amato G, Canonica GW, Salzillo A, Piccolo A, Passalacqua G. Systemic reactions from skin testing: literature review. J Investig Allergol Clin Immunol 2006;16:75-8.
23. Lockey RF, Turkeltaub PC, Olive CA, Baird-Warren IA, Olive ES, Bukantz SC. The Hymenoptera venom study 2: Skin test results and safety of venom skin testing. J Allergy Clin Immunol 1989;84:967-74.
24. Jeep S, Reiprich G, Kunkel G. Yellow jacket allergy. Comparison of skin prick tests and intradermal tests with three different yellow jacket venom extracts. Allergy 1992;47:35-40.
25. Graif Y, Confino-Cohen R, Goldberg A. Reproducibility of skin testing and serum venom specific IgE in Hymenoptera venom allergy. Ann Allergy Asthma Immunol 2006;96:24-9.
26. Goldberg A, Confino-Cohen R. Timing of venom skin test and IgE determination after sting anaphylaxis. J Allergy Clin Immunol 1997;100:182-4.
27. Mosbech H, Christensen J, Dirksen A, Soborg M. Insect allergy. Predictive value of diagnostic tests: a three year follow-up study. Clinical Allergy 1986;16:433-40.
28. Korosec P, Erzen R, Silar M, Bajrovic N, Kopac P, Kosnik M. Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results. Clin Exp Allergy 2009;39:1633-4.
29. Mosbech H. Insect allergy. Allergy 1984;39:543-9.

30. Sturm GJ, Schuster C, Kranzelbinder B, Wiednig M, Groselj-Strele A, Aberer W. Asymptomatic sensitization to Hymenoptera venom is related to total immunoglobulin E levels. Int Arch Allergy Immunol 2009;148:261-4.
31. Rueff F, Przybilla B, Bilo MB Mueller U, Scheipl F, Aberer W et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: Importance of baseline serum tryptase—a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. J Allergy Clin Immunol 2009;124:1047-54.
32. Hauk P, Friedl K, Kaufmehl K, Urbanek R, Forster J. Subsequent insect stings in children with hypersensibility to Hymenoptera. J Pediatr 1995;126:185-90.
33. Blaauw PJ, Smithuis LO. The evaluation of the common diagnostic methods of hypersensitivity for bee and yellow jacket venom by means of an in-hospital insect sting. J Allergy Clin Immunol 1985;75:556-62.
34. Ludolph-Hauser D, Rueff F, Fries C, Schöpf P, Przybilla B. Constitutively raised serum concentration of mast-cell tryptase and severe anaphylactic reactions to Hymenoptera stings. Lancet 2001;357:361-2.
35. Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitkowski M. Basal serum tryptase level correlates with severity of Hymenoptera sting and age. J Investig Allergol Clin Immunol 2007;17:65-9.
36. Bousquet J, Menardo JL, Aznar R, Robinet-Levy M, Michel FB. Clinical and immunological survey in beekeepers in relation to their sensitization. J Allergy Clin Immunol 1984;73:332-40.
37. Antonicelli L, Bilo MB, Napoli G, Farabollini B, Bonifazi F. European hornet (Vespa crabro) sting: a new risk factor for life-threatening reaction in hymenoptera allergic patients? Eur Ann Allergy Clin Immunol 2003;35:199-203.
38. Turkeltaub PC, Gergen PJ. The risk of adverse reactions from percutaneous prick-puncture allergen skin testing, venipuncture, and body measurements: data from the second National health and Nutrition Examination Survey 1976-80. J Allergy Clin Immunol 1989;84:886-90.