Hymenoptera Venom Allergy: Management of children and adults in clinical practice

Brief Title: Management of venom allergic children and adults

Bilò MB1, Pravettoni V2, Bignardi D3, Bonadonna P4, Mauro M5, Novembre E6, Quercia O7, Cilia M8, Cortellini G9, Costantino MT10, Cremonte L11, Rizzini FL12, Macchia L13, Marengo F14, Murzilli F15, Patella V16, Reccardini F17, Ricciardi L18, Ridolo E19, Romano A20, Savi E21, Schiavino D22, Severino M23, Pastorello EA24

1U.O.C. Allergology, Department of Internal Medicine, University-Hospital Ospedali Riuniti di Ancona, Italy
2U.O.C. General Medicine – Immunology and Allergology, Foundation IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy
3U.O.C. Allergology, Ospedale Policlinico San Martino, Genova, Italy
4U.S.D. Allergology Integrated University-Hospital of Verona, Italy
5U.O.S. Allergology Hospital S. Anna Como ASST Lariana, Italy
6Complex Organizational Unit of Allergology, University-Hospital A. Meyer, Firenze, Italy
7High Specialization Unit of Allergology, Hospital of Faenza, AUSL (Local Health Unit) of Romagna, Italy
8Allergy Practice, C.d.S. of Scilla, Reggio Calabria, Italy
9Allergology, O.U. Internal Medicine and Rheumatology, Rimini, Local Health Unit of Romagna, Italy
10Departmental Structure Day Hospital Center, Allergology and Clinic Immunology Local Health Unit of Mantova, Intercompany Allergology ATS Val Padana, Italy
11SS Allergologia ASL AL Alessandria, Italy
12S.S.V.D Allergology Civil Hospitals of Brescia, Department of Clinical and Experimental Sciences University of Brescia, Italy
13School and Chair of Allergology and Clinical Immunology, University of Bari, Italy
14Allergology and Clinical Immunology, A.O.U. Città della Salute e della Scienza di Torino
15U.O.S.D. of Allergology Hospital S.S. Filippo and Nicola, Avezzano (AQ), Italy
16Allergy and Clinical Immunology Division, Department of Medicine, Civil Hospital of
Battipaglia, Salerno, Italy
17SOC Pneumology, Respiratory Physiopathology, integrated University Health Authority of Udine, Italy
18Allergy and Clinical Immunology Unit - University Hospital AOU Policlinico G.Martino, Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy
19Department of Medicine and Surgery, University of Parma, Italy
20Unit of Allergology, Presidio Columbus, Rome, Italy
21Operating Departmental Unit of Allergology, Hospital G. Da Saliceto, Piacenza, Italy
22Complex Operating Unit of Allergology, Policlinico Gemelli – School of Specialization in Allergology and Clinical Immunology, Catholic University, Rome, Italy
23Allergy Unit, San Giovanni di Dio Hospital, Florence, Italy
24Complex Unit of Allergology and Immunology at ASST Grande Ospedale Metropolitano Niguarda - School of Specialization in Allergology and Clinical Immunology at University of Milan, Italy

Corresponding author:
Maria Beatrice Bilò, MD,
Allergy Unit, Department of Internal Medicine, University Hospital of Ancona
Via Conca 71, 60126
Ancona, ITALY
Telephone: +39 071 5963804
E-mail: MariaBeatrice.Bilo@ospedaliruniti.marche.it

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0310
SUMMARY

Hymenoptera venom allergy is an epidemiologically underestimated condition representing an important cause of morbidity worldwide. Preventing future allergic reactions in patients who have developed a systemic reaction is based on the correct management of emergency followed by a correct diagnosis, prescription of adrenaline autoinjectors and, where indicated, specific venom immunotherapy (VIT). Some epidemiological studies highlight the poor knowledge of this disease and the frequent inadequacy of its management. Moreover, they emphasize the importance of such a life-saving treatment as specific immunotherapy. The availability of high quality Hymenoptera venom extracts for diagnostic and therapeutic use has dramatically improved the prognosis and the quality of life of allergic patients. The subcutaneous VIT represents the most effective form of immunotherapy with allergen presently available, with a carry-over effect lasting up to several years after its interruption. This report on the management of children and adults allergic to Hymenoptera venom was drawn up by a panel of Italian experts.

The main objective of this consensus is to review the scientific evidences related to diagnosis, therapy and management of patients allergic to Hymenoptera venom and is aimed to improve the knowledge about this disease and promote good clinical practices. Practical suggestions for a correct diagnosis, prescription of emergency therapy and immunotherapy, as well as strategies for taking care of patients' management are included.

Key words

Adults, Children, Diagnosis, Efficacy, Hymenoptera, Immunotherapy, Management, Safety
RESUMEN
La alergia al veneno de himenópteros es una condición subestimada epidemiológicamente que representa una causa importante de morbilidad en todo el mundo. La prevención de reacciones alérgicas futuras en pacientes que han desarrollado una reacción sistémica se basa en el manejo correcto de la emergencia, seguido de un diagnóstico correcto, la prescripción de autoinyectores de adrenalina y, en el caso de estar indicada, la prescripción de inmunoterapia específica con veneno (VIT). Varios estudios epidemiológicos destacan el escaso conocimiento de esta enfermedad y un frecuente tratamiento insuficiencia. Además, enfatizan la importancia de la inmunoterapia específica, un tratamiento que puede salvar la vida del paciente. La disponibilidad de extractos de veneno de himenóptera de alta calidad para uso diagnóstico y terapéutico ha mejorado drásticamente el pronóstico y la calidad de vida de estos enfermos. La VIT subcutánea representa la forma más efectiva de inmunoterapia con alérgeno actualmente disponible, con una eficacia persistente que dura hasta varios años después de su interrupción. Este consenso sobre la evaluación clínica tanto de niños y como de adultos alérgicos al veneno de himenópteros ha sido elaborado por un panel de expertos italianos. Su objetivo principal es revisar la evidencia científica disponible en el diagnóstico, la terapia y la evaluación clínica de los pacientes alérgicos al veneno de himenópteros con el propósito de mejorar el conocimiento sobre esta enfermedad y promover buenas prácticas clínicas. Se incluyen sugerencias prácticas para un diagnóstico correcto, la prescripción de terapia de emergencia e inmunoterapia, así como estrategias para el manejo de los pacientes.

Palabras clave: Adultos; niños; diagnóstico; eficacia; Himenópteros; inmunoterapia; tratamiento; seguridad.
Introduction

Hymenoptera venom allergy is an epidemiologically underestimated condition representing an important cause of morbidity worldwide. Hymenoptera venom allergy is an epidemiologically underestimated condition representing an important cause of morbidity worldwide. Mortality rate is low; however, underestimates are common, with many sting fatalities being misdiagnosed. Preventing future allergic reactions in patients who have developed a systemic reaction is based on the correct management of emergency followed by diagnosis, prescription of adrenaline autoinjectors and, where indicated, specific venom immunotherapy (VIT). Some epidemiological studies highlight the poor knowledge of this disease and the frequent inadequacy of its management [1]. Moreover, they emphasize the importance of such a life-saving treatment as specific immunotherapy.

The availability of high quality Hymenoptera venom extracts for diagnostic and therapeutic use has dramatically improved the prognosis and the quality of life of allergic patients. The subcutaneous VIT represents the most effective form of immunotherapy with allergen presently available, with a carry-over effect lasting up to several years after its interruption.

This report on the management of Hymenoptera venom allergy was drawn up by a panel of Italian experts.

Objectives and work methodology

The main objective of this consensus is to review the scientific evidences related to diagnosis, therapy and management of patients allergic to Hymenoptera venom and is aimed to improve the knowledge about this disease and promote good clinical practices. Practical suggestions for a correct diagnosis, prescription of emergency therapy and
immunotherapy, as well as strategies for taking care of patients' management are included. The data concerning the various topics treated in this report were obtained from studies published in the literature in both English and Italian language and were collected by searching MEDLINE and EMBASE databases. GRADE system has been used for translating research results into recommendations based on scientific evidence [Box 1] [2]. All recommendations which received agreement by greater than or equal to 90 % of the Authors were included in this report. The panel of experts was constituted by physicians with high experience in hymenoptera venom allergy and working in one of the main allergy centres. Some centres started with VIT in the early 80s.

HYMENOPTERA VENOM ALLERGY: EPIDEMIOLOGY AND CLINIC

Hymenoptera

The insects of the order of Hymenoptera comprise some aculeate species whose venom can trigger allergic reactions in humans, which may span from relatively mild reactions to fatal anaphylaxis [3]. In Europe Hymenoptera causing allergic reactions belong to the Apidae and Vespidae families or, sporadically, to Formicidae (Formica rufa) [4] and Myrmicidae (Solenopsis invicta) [5], which are widespread in the North and Central America and in Australia.

The Apidae family includes the subfamilies Apinae (Apis mellifera) and Bonbinae (Bombus terrestris, agrarum, medics), while the Vespidae family is composed by the Vespinae subfamily, including genera Vespula (germanica, vulgaris), Dolichovespula (maculata, arenaria, saxonica, media) and Vespa (crabro, orientalis velutina nigritorax) and the subfamily Polistinae including genus Polistes (dominula, gallicus).

The bees and the vespids of genus Vespula are widely spread also in the Far Northern regions of Europe. In Southern Europe, apart from Vespula, a frequent cause of allergic
reactions is also represented by hornets (genus Vespa), including the most widespread species *Vespa crabro* and some species of *Polistes*, such as *Polistes dominula* [6]. The genus Dolichovespula has a more limited diffusion and can be considered similar to *Vespula* from an allergological point of view.

In 2005 *Vespa velutina nigritorax* from South East Asia, belonging to the genus *Vespa*, was detected in the South of France. The *Vespa velutina* is a predator of bees and is rapidly spreading from France to neighboring countries. Some anaphylactic reactions have been described after *Vespa velutina* stings with a variable degree of cross-reactivity with other vespids [7].

Allergy to bumble bees, due to its low aggressiveness, concerns a limited number of subjects, in particular professionally exposed individuals [8], and it should therefore be investigated on the basis of a specific anamnestic suspicion, provided that a suitable extract is commercially available for diagnosis. Immunotherapy with honeybee venom alone may be sufficient in non-professionally exposed bumblebee-allergic patients with bee venom primary sensitization, whose reaction is most likely due to cross-reactivity. In occupationally exposed patients, who are frequently stung by bumblebees, purified bumblebee venom for immunotherapy, when available, is recommended [9].

The recognition of the stinging insect, as hard as it is, remains crucial in the management of the allergic reactions being an integral part of the diagnostics flow towards the choice of specific immunotherapy; thus, information on behavior and morphological characteristic of the clue insects allow the clinician to figure out the correct clinical history and the diagnosis. *Apis mellifera* has a characteristic serrated sting which remains stuck into the tissues of the victim together with the venom sack. The bee dies by self-evisceration when flying away from the victim. The vespids and other Apids (bumblebees), instead, have smooth stings, which can be extracted from their victims allowing them to sting several times consecutively.
Epidemiology

Depending on living environment and type of activity, 56-94% of the adult population is estimated to have been stung by a hymenopter at least once in a lifetime, being in Europe a bee in about one third of cases [10]. As a consequence, the development of specific IgE toward one or more allergens of venoms can occur as an ancestral defence response against the toxic effects of venom [11]. Such a response may be favored by atopic diathesis and by genetic factors and may correlate with high level of total IgE [10,12].

The prevalence of asymptomatic sensitization is estimated to range from 9.3 to 40.7% in the adult population with higher proportions in case of high exposure, such as for example in beekeepers (30-60%) [13,14].

Epidemiological studies report a large variability of the prevalence of allergic reactions: being repeatedly exposed to stings (studies on bee keepers) increases the prevalence of large local reaction (LLR) up to 38% [15] and of systemic reactions to 30%-45% [16,17].

In Europe the prevalence of systemic reactions in the adult general population is 0.3-8.9% [10,18] and raises to 14-32% among beekeepers [13]. Taking into account the studies carried out on anaphylaxis as a whole, Hymenoptera stings represent the cause of 7.3-59% of cases depending on the investigated populations and is more frequent in adults [15].

According to data from the European Anaphylaxis Registry, out of 3333 diagnosed cases, Hymenoptera venom allergy was the most frequent cause of severe reactions in the adult population (48.2%) [19].

Data from the emergency departments of several parts of the world show that Hymenoptera venom allergy is responsible for 1.5-34% of anaphylactic reactions with lowest prevalence in urban hospitals [10].

Recent Italian studies on cases of anaphylaxis reported directly by emergency departments
to allergy centres for diagnostic assessment have shown that Hymenoptera venom allergy is the most frequent cause (42-70% of cases) [20,21].

In different countries Hymenoptera venom allergy is responsible for about 20% of the total cases of fatal anaphylaxis [10], due to shock with multiple organ failure within 10-15 minutes after sting and, in a quarter of cases, due to glottis edema [22]. Overall, the incidence of mortality in the various European countries is between 0.03/million/year in Italy and 0.48 in France. In Italy ISTAT data in the period 1994-2003 reported 94 deaths [15]. Mortality data are generally underestimated as deaths are likely to be attributed by mistake to other causes, in particular to cardiac disorders [10].

Since 40% of fatal anaphylaxis occurs as first reaction to Hymenoptera venom, the possible risk factors which may cause a transition from an asymptomatic sensitization to a more severe clinical manifestation need careful evaluation [10,23-26].

**Clinical Aspects**

Hymenoptera venom is a mixture of different components including bioactive molecules such as histamine, serotonin, tyramine, catecholamines, low molecular weight peptides (including mastoparans, kinins, chemotactic peptides), high molecular weight proteins (including phospholipase, hyaluronidase, mellitin, antigen 5) which differ in the different species and can act as allergens and, in some cases, can cause toxic reactions.

From the clinical point of view, we can distinguish local reactions, large local reactions, systemic allergic reactions, toxic systemic reactions, unusual reactions.

Local reactions in most cases consist of itching, erythema and edema of limited extension; they are transient, normal consequences of the vasoactive and inflammatory action of some venom components. In the event of allergy, more severe large local reactions may occur, characterized by delayed and prolonged inflammation and edema increasing within 24-48
hours and resolving in 3-10 days, with an extension exceeding an average of 10 cm in diameter.

The Anaphylaxis Guidelines of the World Allergy Organization [27] and of the European Academy of Allergy and Clinical Immunology (EAACI) [28] have established the clinical criteria for the diagnosis of anaphylaxis, confirming the proposal of the Second Symposium on the definition and management of anaphylaxis: summary report - Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network Symposium [29].

Various classifications of the degree of severity of SRs have been proposed, those of Mueller [30] and Ring [31] being the most frequently referred to. Both classifications have limitations: Mueller's does not take into account the possible absence of cutaneous symptoms and that an isolated cardiovascular shock might be the only allergic sting-induced manifestation [29], while Ring's is almost entirely focused on the cardiovascular collapse, which is considered more severe than respiratory impairment.

The classification of mild, moderate and severe reactions according to Brown [32] can also be adapted to systemic allergic reactions to Hymenoptera venom [33]. EAACI has recently proposed a simplification of the severity criteria of acute allergic reactions, dividing them into local (grade 1) and systemic (grades 2 and 3) [34].

Skin symptoms are the most frequent (80%) and represent the only manifestation in 15% of cases of systemic reactions of adults. In some cases, the onset of chronic urticaria and cold urticaria after sting was reported, generally without immediate reaction and with a risk for systemic reactions to re-sting unascertained. Almost 50% of systemic reactions includes respiratory symptoms (angioedema of upper airways, bronchospasm). Symptoms and signs of hypotension may occur in over 60% of adults, in half of the cases with loss of consciousness. Cardiac involvement during anaphylaxis can cause bradycardia,
arrhythmias and acute coronary syndromes, compatible with Kounis’s syndrome [35,36]. It can be also secondary to a decreased venous return in its turn due to histamine induced vasodilatation and permeabilization. Gastrointestinal symptoms (abdominal pain, nausea, vomiting, and diarrhea) and uterine cramps, with possible miscarriage, may occur. Neurological symptoms (e.g., convulsions) may also occur. Biphasic anaphylaxis, characterized by recurrence of anaphylactic symptoms within 4-12 (exceptionally 72) hours [37] after resolution (without re-exposure) was reported in 0.4%-14.7% of the cases [38].

Toxic systemic reactions are caused by the action of venom components with enzymatic activity and organ-specific toxicity; they usually occur after multiple simultaneous stings (from several tens to several hundreds). Toxic effects occur in hours to days and consist of rhabdomyolysis, intravascular hemolysis, coagulation disorders, hepatic damage, acute renal failure; fatal cases are sporadic [39].

Unusual reactions are rare, due to a toxic or non-IgE-mediated immunological mechanism, in some cases to autoimmunity; they can also occur after a single sting, within hours to days. They include serum-like sickness manifestations, manifestations of central nervous system (acute encephalopathy, Guillain-Barré syndrome, myasthenia, peripheral neurites), hematological reactions (thrombocytopenic purpura, -Schönlein-Henoch purpura, hemolysis, coagulation disorders), muscle (rhabdomyolysis), renal (acute renal failure due to interstitial nephritis or tubular damage, nephrotic syndrome) and respiratory reactions (alveolar hemorrhage) [40]. Episodes of metrorrhagia after a bee sting have been described among the unusual reactions [41].

**Pediatric aspects**

The prevalence of asymptomatic sensitization is 3.7% in an Italian pediatric case study [42]. The prevalence of large local reactions is reported between 0.9% [43] and 20.8% [44],
the prevalence of systemic reactions is globally below 1% [15,42].

According to the European Anaphylaxis Registry, Hymenoptera venom allergy is the second cause of severe reactions in the pediatric age (20.2%), after food allergy [19].

Risk factors for severe systemic reactions after Hymenoptera stings in children were evaluated by Graif [45] in a population of adolescents aged 13-14 years. Atopic children had a significantly higher rate of severe reactions than non-atopic (36.9% vs 24.8%). Therefore asthma, allergic rhinitis and atopic eczema should be considered risk factors for reactions of any severity; moreover, the severity of the reaction relates also to the severity of asthma.

Atopy was confirmed as a risk factor for severe reactions also in a more recent study [46]. However, this finding should be confirmed in larger populations of children.

In children systemic reactions mostly affect skin and rarely cardio-circulatory system. Skin symptoms are the only clinical manifestation in 60% of cases [47]. Children have a favorable prognosis regarding re-sting, both in studies with sting challenge [48], and field sting [49,50].

TREATMENT OF ACUTE REACTION

In hospital setting

The treatment of anaphylactic reaction in hospital setting shall comply as much as possible with Guidelines; After discharged the patient should be referred to an allergy specialist and prescribed adrenaline autoinjectors [51-53]. Regarding post-anaphylaxis monitoring, WAO Guidelines indicate a minimum of 8-10 hours for the risk of late anaphylactic reactions [27]. American Guidelines [54] suggest individualizing this period, EAACI Guidelines [28] advise a minimum duration of 6 to 8 hours for patients with respiratory symptoms and 12-24 hours
in the event of hypotension or collapse.

The panel of experts believe that the patient, after receiving the appropriate therapies and obtained the resolution of clinical picture, should be kept under observation and monitored for at least 6-8 hours up to 24 hours depending on severity and characteristics of the reaction at onset, comorbidity and risk factors (strength of recommendation D). The duration of this period depends sometimes on internal regulations of individual hospitals.

The management of anaphylaxis in hospital setting requires general measures and the administration of specific medicinal products [27].

General measures include: a) monitoring of vital parameters; b) positioning of the patient in Trendelenburg position (supine with legs raised 10°-15°) or, in case of vomiting, on lateral right side; if the patient is pregnant, it is recommended, in supine position, to gently dislodge the fetus to the left so as to decompress the inferior vena cava and thus improve the venous return to the heart; if the woman is laid on one side it should be the left; c) rapid cannulation of a peripheral venous access with cannula needle of high gauge (at least 18 G); d) rapid intravenous administration of isotonic saline solution (plasma expanders should be avoided for the risk of mast cell degranulation); e) administration of oxygen (if necessary); in case of pregnancy oxygen should be administered to avoid hypoxemia of fetus (4 litres/minute using nasal prongs); f) continuous clinical and instrumental monitoring of the patient, by measuring arterial blood pressure, heart rate (bradycardia in hypotension seems to have a negative prognostic value), peripheral oxygen saturation.

The specific medicinal products used in the management of anaphylaxis are:

- **Adrenaline**: is the treatment of choice for anaphylaxis regardless of the presence of shock (strength of recommendation C) [27-29,55,56]. It slows the progression of symptoms and can prevent the development of fatal or biphasic reactions (strength of recommendation C) [57,58]. If a correct dosage is administered, it can be used, without absolute
contraindications, in pediatric and geriatric populations and in cardiopathic patients, [28,59,60], except for some cardiac pathologies such as for example long QT syndrome (in this case, the administration should be performed with extreme caution, in case of real need and in the presence of the cardiologist).

Adrenaline remains the drug of choice for the treatment of anaphylaxis also for pregnant women (strength of recommendation D) [61-63]; in fact, ephedrine may have a lower risk of uterine contractions but, if inefficacious, it may allow escalation of the anaphylactic reaction with the consequent risks.

Adrenaline should be administered intramuscularly in the lateral thigh (vastus lateralis muscle), at a dose of 0.01 mg/kg of a 1/1000 solution, with a maximum dose of 0.3 mg in children and 0.5 mg in adults [27]. The dose may be repeated after 5-15 minutes if necessary (strength of recommendation B) [27,59].

The intravenous administration should be reserved to the most severe cases, with imminent danger of life for cardio-circulatory collapse (strength of recommendation D) [56,64]. The infusion should be stopped 30 minutes after clinical stabilization. Table 1 describes the modalities and the concentrations of adrenaline intravenously.

- **Dopamine**: to be used if it is not possible to maintain a stable circulatory function with adrenaline. The dosage is 5-15 µg/Kg/min. Table 2 shows the hourly rate of infusion in syringe pump for the desired dosages based on body weight.

- **Antihistamines**: the use of anti-H1 is recommended only for the treatment of skin symptoms (strength of recommendation B) [28,65,66]. There are no controlled studies to support the use of antihistamines for the treatment of anaphylaxis [67]. The intravenous administration has the advantage of acting more quickly, but it should be done very slowly to avoid side effects (including hypotension). The suggested dosage is generally 10 mg of chlorpheniramine in adults and 2.5-5 mg in children [27].
The concomitant administration of anti-H2 antihistamines has not proved to have greater therapeutic efficacy and therefore it is not recommended in the various guidelines.

- **Glucocorticoids**: are used for the control of bronchospasm and prevention of biphasic reactions [28,54,59,65], even if there are no controlled studies to confirm their effectiveness in the treatment of acute anaphylactic reaction (*strength of recommendation D*) [68]. Hydrocortisone 200 mg intravenously in adult (in the child up to 100 mg) or methylprednisolone 50-100 mg intravenously in adult (in child 1 mg/kg, maximum 50 mg) [27] are recommended.

- **Glucagon**: exerts both positive inotropic and chronotropic effects for activation of the adenyl cyclase activity independent from beta-receptor. It is sometimes needed in patients taking a beta-adrenergic blocker who have hypotension and bradycardia and who do not optimally respond to adrenaline. Glucagon can be administered in adult patients intravenously at a dose of 1 mg as an initial IV bolus, repeatable every 5 minutes, increasing the dosage to 3-5 mg if necessary. The administration for continuous infusion in syringe pump should be carried out at the dose of 1-5 mg/hour (*strength of recommendation D*) (Table 3). It should be pointed out that it is an off-label drug for the therapy of anaphylaxis. Glucagon can lead to severe vomiting and hyperglycemia [69,70].

- **Desmopressin**: literature reports the use of desmopressin for the treatment of anaphylactic shock unresponsive to adrenaline (*strength of recommendation D*) [71,72].

- **Bronchodilators**: short-acting formulations via inhalation route (e.g. salbutamol) are to be preferred.

**Self-treatment of the patient with anaphylaxis**

All patients with a history of anaphylactic reaction should be provided with adrenaline autoinjector to be injected in vastus lateralis muscle [27,28,73]. The autoinjectors currently
available can differ in several countries [74]. In obese or overweight patients, the reduced length of the needle does not always ensure the intramuscular administration [75,76], therefore the patient should be advised to press well the autoinjector on fatty thigh, to compress it and allow the penetration of the needle into the muscle.

A study has compared three adrenaline autoinjectors, two “cartridge-based” (EpiPen and Jext) and one “syringe-based” (Anapen) for penetration depth in ballistic gelatin [77]. For the two systems “cartridge based” the mean maximum injection depth in gelatin within 10 seconds was 29.68 mm (SD 2.08) for EpiPen and 28.87 mm (SD 0.73) for Jext; for the system “syringe-based” (Anapen) was 18.74 mm (SD 1.25). “Cartridge-based” systems therefore reached a depth double the length of the needle. In the same study it was also assessed that the average height of the adipose tissue in 50 females was 14.8 mm.

Comparing the robustness and performance of these three devices, it was concluded that “cartridge-based” systems are more robust and ensure greater speed, validity, correctness of the dose, accuracy of the site of administration compared to the “syringe-based” system. However, a recent study has shown that the bioavailability of adrenaline administered by “syringe-based” autoinjector is not affected by needle length [78].

In the paediatric population, considered the fixed dosages of the autoinjector, there is a risk of administering a lower or higher dose, depending on body weight [59,79]. In children weighing 15-30 kg, a lower dose should be used if the anaphylactic reaction has not been severe, an adult dose if the anaphylaxis has been severe or in case of concomitant bronchial asthma (risk factor for fatal anaphylaxis) [80].

Table 4 shows symptoms and signs indicative of an anaphylactic reaction for a correct use of the adrenaline autoinjector; this table can be provided to the patient during training to use the autoinjector.

Although many patients are afraid to use adrenaline autoinjector for fear of side effects [81],
with the exception of the known onset of tachycardia, tremors and peripheral vasoconstriction, there are no reports of significant adverse events [82].

Even if adrenaline remains the first choice drug in anaphylactic reaction, the patient with mild systemic reactions (e.g. only hives) may also take as self-treatment oral steroid (for instance methylprednisolone tablets 16 mg = 4 tablets) and a double dose of last-generation antihistamines.

**Indications for the prescription of adrenaline autoinjectors**

Adrenaline autoinjector should be prescribed to the following categories of patients [28,33,73,74]:

- Children and adults with systemic reactions more severe than systemic skin reaction or with a high risk of re-exposure to sting (e.g. beekeepers), before VIT: *Level of evidence IV, strength of recommendation C*.

- Children and adults undergoing VIT, but with risk factors for incomplete clinical protection (very severe onset reaction, adverse reactions during immunotherapy, lack of sting protection during VIT, bee venom allergy): *Level of evidence V, strength of recommendation D*.

- Children and adults who have discontinued VIT, but presenting risk factors for incomplete clinical protection (e.g. particularly severe pre-VIT systemic reaction, systemic reaction caused by VIT, lack of protection during VIT): *Level of evidence V, strength of recommendation D*.

- Children and adults with elevated levels of serum mast cell tryptase or mast cell disorders, and a history of systemic reaction to Hymenoptera sting, independently from VIT: *Level of evidence IV, strength of recommendation D*.

- Children and adults who discontinued VIT, despite suffering from mast cells disorders
and/or elevated levels of serum mast cell tryptase: Level of evidence IV, strength of recommendation C.

According to European Guidelines the prescription of two adrenaline autoinjectors is recommended in patients suffering from mast cell disorders and/or elevated levels of serum mast cell tryptase, in patients with a history of very severe anaphylactic reactions who required the administration of multiple doses of adrenaline or who cannot quickly access to hospitals [28]. Based on the data currently available in the literature [83], the group of experts considers basal tryptase levels above 7.95 μg/L as high in those patients with a history of anaphylactic reaction caused by Hymenoptera sting with loss of consciousness, without cutaneous/mucosal involvement.

The group of experts also suggests prescribing two adrenaline devices to obese subjects, as the injection might not reach the muscle and therefore could be less effective.

Regarding LLR, the risk of following systemic reaction at the moment is not considered so high as to require the prescription of adrenaline [74]. Nevertheless, the Italian experts do not rule out the possibility of prescribing adrenaline to patients at risk of multiple stings (e.g. beekeepers) and to those who have developed a single LLR, since in these subjects the risk of a subsequent systemic reaction to a re-sting cannot be completely excluded compared to patients who have already shown repeated LLR [84,85].

EMA provisions on adrenaline autoinjectors

After evaluation of all available data, the European Medicines Agency (EMA) confirmed that intramuscular administration is the most indicated route for obtaining a rapid response in the treatment of anaphylaxis [86].

EMA observed that the correct administration of adrenaline by autoinjectors is affected by several factors such as needle length, thickness of subcutaneous fat, mode of operation of
the autoinjector (whether or not spring-loaded and/or cartridge-based), angle with which it is placed on the skin, force used to activate it, patient's ability to follow the instructions properly.

Healthcare professionals are in any case recommended to prescribe two autoinjectors which patients shall always bring with them, and to instruct the patient to use the autoinjector through educational material and practical training.

**DIAGNOSTIC CRITERIA**

Diagnosis is based on the classification of the type of reaction, confirmation of the IgE-mediated pathogenesis and identification of the stinging insect. On this basis, clinical history and results of *in vivo* and *in vitro* tests are crucial [3,87].

History includes the description of the symptoms and of the course of the reaction (possibly documented by a medical report), the number of stings, the characteristics of the culprit insect (where possible) and the identification of specific risk factors for the severity of reaction [3].

It may be useful to show the patient an entomological notice board to facilitate the identification of the stinging insect; 73% of the patients allergic to *Vespula* spp accurately identify this kind of Hymenoptera in the board [88].

Since it is possible to document a sensitization to the venoms in 10-30% of subjects with negative history, only patients with a history of previous systemic reaction [3,33,87,89] should be investigated. Table 5 shows the indications for execution of diagnostic tests.

In patients with a history of LLR, skin tests (as well as specific IgE determination) may be considered as optional, at the discretion of the clinician in specific cases, like in patients at a greater risk of re-sting with recurrent and bothersome LLRs (e.g. beekeepers, farmers) who could benefit from immunotherapy [90].
Skin tests are the gold standard for diagnosis and should be carried out at least two weeks after the last sting, to exclude a false negative response during the refractory period [3,33,87]. As this period may be even longer, in case of negative tests in subjects with a suggestive history, tests should be repeated after 1-2 months. Vice versa some patients’ sensitization can be demonstrated only during the first week after the reaction [91].

European Guidelines suggest to perform skin tests gradually, that is prick tests first followed, if negative, by intradermal tests [3,33,87]. Intradermal tests should be performed even in case of positive prick test to identify correctly the cutaneous end-point which will be useful in VIT follow-up. The correct execution of skin tests with Hymenoptera venom is of crucial importance, both for a correct diagnosis and for VIT monitoring [92]. In particular, intradermal tests should be carried out by the administration of 0.02 mL of the allergenic extract into the dermis, causing the development of a wheal approximately of 3 mm in diameter. The reading should be performed after 15-20 minutes; the positivity is documented by an increase of at least 3 mm of the average diameter of the initial wheal, with associated erythema. To allow comparison of results, a morphological score should be used, which consists in drawing, on transparent cellophane, the area injected and the area of the reaction after 15-20 minutes of time [93].

The prick test is carried out at the 100 µg/mL concentration. The intradermal reactions can start from very low concentrations, according to the symptoms presented by the patient; concentrations ranging from 0.001 to 1 µg/mL are normally used.

The sensitivity of the prick test is lower than the one of the intradermal test. In a study performed on 301 Vespula spp venom allergic patients, prick test identified 49% of cases, while the combination between prick and intradermal reaction allowed diagnosis in 94% of cases [94]. Intradermal test with non-dialysate venoms can be irritating at a concentration higher than 1 µg/mL [95]. In Europe standardized venoms of Apis mellifera, Vespula spp.
and *Polistes* spp., *Vespa crabro* are currently available; the venoms of *Vespula* and *Polistes* consist of a mix of clinically relevant species, respectively (*Vespula* spp.: *Vespula vulgaris*, *V. flavopilosa*, *V. germanica*, *V. maculifrons*, *V. pennsylvania*, *V. squamosa* - *Polistes* spp [American]: *Polistes annularis*, *P. exclamans*, *P. fuscatu*, *P. metricus*). Because of low cross-reactivity between European and American *Polistes* venoms [96] extracts of *Polistes dominula* are now available for both diagnosis and VIT [97]. On the other hand, a high cross-reactivity between *Vespula* spp venom and *Vespa crabro* has been confirmed [98]. A recent study [99] suggests that, in patients with proven reaction to *Vespa crabro*, VIT with *Vespa crabro* venom may have a higher safety profile.

Skin tests with venoms are generally safe, even in patients with mastocytosis [100, 101]. A study has highlighted safety even if the tests are carried out simultaneously at different concentrations [102]. The panel of Italian experts, considering that available data are insufficient, recommends a preliminary step where the same concentration of more venoms is simultaneously used for skin testing. Only after reading the reactions to this first set, a higher concentration should be used. This caution is to be maintained specially in patients with severe anaphylactic reaction or suffering from mast cells disorders.

The dosage of the total IgE may be useful for a correct interpretation of specific IgE values, especially if they are very low [103]. In the event of very high levels, the presence of a concomitant pollinosis should be checked.

The presence of serum specific IgE can be detected immediately after sting, even if the best period for their determination is 1-4 weeks after sting [3].

The sensitivity of serological tests using whole extracts is generally lower than that of skin tests. In general, *in vitro* tests for the search of specific IgE toward the whole extract of venom can be negative in up to 20% of patients with positive skin tests, whereas approximately 10% of patients with negative skin test are positive at *in vitro* test. For this
reasons Guidelines suggest performing both tests [3,33,87,104].

The sensitivity of serological tests for *Vespula* spp. is lower than the one for bee venom; a sensitivity ranging between 98% and 100% for the bee [105,106] and between 83 and 97% for the Vespula [105,106] has been reported. A new *in vitro* method enriched with recombinant allergen Ves v 5 showed a greater sensitivity compared to traditional methods [94]. Furthermore, it has been recently hypothesized that negative skin tests of *Apis mellifera* extract may be due to a minor presence or even absence of some allergens in diagnostic and therapeutic extracts [107]. It is to be noted that the values of serum specific IgE to *Vespa crabro* venom may vary according to laboratory method used.

Diagnosis is complicated by the sensitizations to multiple venoms in patients who have not identified the stinging Hymenoptera. The double positivity to venom of *Apis mellifera* and *Vespula* spp. is found in 25-40% of cases and may be due to: 1. double sensitization; 2. cross-reactivity between epitopes present in both venoms (hyaluronidase; *Api m 5* and *Ves v 3*; *Api m 12* and *Ves v 6*); 3. cross-reactive carbohydrates (CCD). The availability on the market of some major allergens expressed in recombinant form allows to implement a component resolved diagnosis (CRD) [103,108].

Bee venom allergic patients often have a broad sensitization profile. *Api m 1*, the most relevant allergen of bee venom, is not sensitizing in up to 43% of cases [109]. The combination of 2 allergens (*Api m 1* and *10*) allows diagnosis in 86.8% of cases; the combination of 6 allergens (*Api m 1-5, Api m 10*) has a sensitivity of 94.4% [109]. The following recombinant allergens are currently marketed: *rApi m 1, rApi m 2, rApi m 3, rApi m 5, rApi m 10*. Patients with *Vespula* spp. venom allergy are sensitized mainly to *Ves v 1* and *Ves v 5*. The combined search of specific IgE toward these two recombinant allergens allows the identification of 92-94% of patients allergic to *Vespula* [110,111].

In Southern Europe double *Vespula*-Polistes sensitization is more frequent than Apis-
Vespula [112], and the cross-reactivity between allergens of two species often poses diagnostic difficulties [113,114]. In case of difficult interpretation between Vespula and Polistes sensitizations, in clinical practice the use of Ves v 5 and Pol d 5 seems to be helpful provided that the difference of specific IgE levels between the two molecules is particularly significant, with at least double values of one recombinant over the other [114-116]. Also phospholipases (Pol d 1/Ves v 1), where available, have proved to be useful to discriminate the probable sensitizing species in Vespula/Polistes sensitized patients [114]. A new major allergen of the venom of Polistes dominulus, Pol d 3 (dipeptidyl peptidase IV) has recently been identified, but it was found cross-reactive with both Apis and Vespula venoms [117]. IgE to CCD can explain multiple in vitro positive results; serum determination for CCD (bromelain or MUXF3) allows greater diagnostic accuracy [105]. Polistes venom is CCD-free and therefore is not affected by such a cross-reactivity [118].

Figures 1 and 2 suggest the diagnostic algorithm in case of double positive results Apis-Vespula and Vespula-Polistes.

In summary, CRD may discriminate between primary sensitization and cross-reactivity in patients with double positivity of diagnostic tests with whole extracts, allowing the specialist to choose the most suitable venom for VIT, avoiding treatment with double VIT. However, the decision should relay not only on CRD results but also also taking account the severity of the reaction and the patient’s general health condition. CRD may also help in the diagnosis of patients with a history of systemic reaction and negative standard diagnostic tests [119,120].

Another method to distinguish the double sensitization from cross-reactivity is CAP-inhibition, although it may be relatively expensive and difficult to interpret [103]. Its use, where available, appears to be very useful in case of double Vespula-Polistes co-sensitization, when CRD does not suffice to discriminate the different possibilities [114,115].
Among blood-cell based tests, the Basophil Activation Test (BAT) is the most widely used in Europe for diagnostic purposes, in selected situations. If performed in highly specialized laboratories, it can identify approximately two thirds of patients with positive history and negative skin and serological tests [121]. BAT is also recommended in patients with double positive results and inconclusive results of in vivo or in vitro tests with recombinant allergens [119]. Since BAT results are influenced by the presence of venoms CCD, using CCD-free recombinant allergens allows greater diagnostic accuracy [119,122]. The role of BAT as a diagnostic tool in patients with mastcell disorders and negative venom-specific IgE and skin test results is still controversial [123-126]. Sting challenge with a live insect should not be used for diagnostic purposes, due to the risk of systemic, potentially severe reactions and low negative predictive value [127].

In the presence of systemic reactions, basal serum tryptase levels should always be determined, as adults affected by mast cells disorders and/or elevated basal tryptase levels have a significantly greater risk of developing severe reactions to Hymenoptera stings [16,128].

Moreover, it should be pointed out that patients should be investigated for mastocytosis even in the absence of cutaneous manifestations compatible with mast cell pathology and increased tryptase levels, in case a severe anaphylactic reaction with syncopal episode without urticaria and/or angioedema and a REMA score ≥2 [129]. High basal serum tryptase are not pathognomonic of mastocytosis and can be found also in hematological diseases, especially of the myeloid lineage, end-stage chronic renal failure, onchocercosis in treatment, abdominal aortic aneurysm (two cases with anaphylaxis to Hymenoptera venom were reported) [130].

Table 6 shows some practical considerations to be considered in the diagnostic flow chart for Hymenoptera venom allergies.
Pediatric aspects
Diagnostic tools are not different from those used in adults; also in children, the degree of skin sensitization does not correlate with the severity of the reaction [131].

SPECIFIC IMMUNOTHERAPY

Definition and mechanisms of action
VIT is the therapy of choice for subjects who developed a systemic reaction after Hymenoptera sting, since it can induce tolerance to venom [33,73,132-134].
VIT consists of an “induction” phase and a “maintenance” phase.
The induction phase implies the subcutaneous administration of increasing doses of the extract of the stinging insect venom up to the protective dose, *i.e.*, 100 μg; to this aim several protocols of different length can be used.
The maintenance phase implies the administration of fixed amounts of venom at regular time intervals to maintain the tolerance status.
The mechanisms of action of immunotherapy are multiple and impact both at early and late stages. They include the increase in specific IgG1 and IgG4 levels [135-138], the cytokine shift characterized by reduction of IL-4 and IL-5, and increase of IFNγ [139-141], the reduced expression of adhesion molecules [142], lymphocyte down-regulation [143], reduction of mast cell and basophil activation [144,145], immunomodulation by IL-10 [137,146-148], induction of regulatory T cells [131,149,150].

Indications
VIT is indicated in the following circumstances: a) children and adults with a systemic reaction involving other apparatus besides the skin [90]. b) systemic skin reactions at high risk of exposition and/or impairment of quality of life [151] in adults [90]. c) patients with
clonal mast cell disorder and a history of a systemic reaction [152], even though sensitization can be weak or sometimes transitory.

Regarding children with cutaneous-mucosal reactions, please refer to the relative paragraph.

VIT is not generally indicated in case of LLR, as the risk of evolution in systemic reactions is low (2-7%) [89,153,154], especially if the LLRs are repeated [84,89]. As clinical efficacy of VIT has been demonstrated in reducing the extent of consecutive LLRs [155,156], its use is not contraindicated in patients with recurrent and severe LLRs. b) in unusual reactions (i.e. serum-like sickness manifestations, manifestations of central nervous system, hematological, muscle and renal reactions), whose mechanism of action is poorly known [73].

**Clinical efficacy**

Specific subcutaneous immunotherapy for Hymenoptera venom is the only treatment, able to protect patients from systemic reactions after subsequent stings [90]. Numerous studies evaluated VIT efficacy, both with “sting challenge” and “field sting”. In particular, in a recent Cochrane Review the percentage of non-protection was 2.7% in the treated patients vs. 39.8% in the patients not undergoing immunotherapy [156]. Regarding Vespid venom, the protection is between 91-96%, while for bee venom it ranges between 77% and 84% [14,90,157-167]. Studies performed on European populations, including Italian cases, show that about 70% of treated patients are allergic to Vespids [164,166]. The different methods of preparation of the extracts do not affect their protective capacity; the efficacy of purified aqueous and aluminium hydroxide adsorbed preparations (so-called “depot” preparation) is in fact comparable [168].

As for VIT with *Polistes* venom, the use of *Polistes dominula* venom to treat European
patients should be preferred due to reports indicating a lack of protection by American Polistes venom extracts [6,169]. However, a recent study [170], while confirming the incomplete in vitro cross-reactivity, did not detect differences in clinical protection between VIT with mixture of American Polistines and VIT with Polistes dominulus after “field sting”. Further studies are necessary to confirm these data.

Table 7 describes known risk factors for reduced efficacy of VIT.

**Protocols**

Over the years, various induction protocols have been proposed with the aim of reducing the incidence of side effects while rapidly achieving clinical protection and favouring patient compliance. According to the chosen protocol, maintenance dose may be reached in a few weeks, in a few days or in a few hours [33,73,171]. Conventional and “clustered” protocols do not significantly differ from one another as far as safety is concerned [172]. The ultrarush induction protocols have proved effective [173-175], inducing early changes in immunological parameters associated to VIT efficacy (IgE, IgG₄) [176]. To increase patient compliance with VIT, immunotherapy protocols can be managed with a certain flexibility, for example by switching from an aqueous extract to a depot extract by the same manufacturer, without any impact on efficacy and safety [177].

VIT starting dose is between 0.001 µg and 0.1 µg; however, treatment can be initiated safely from 1-5 µg of venom using a rush protocol both in adults and in children [172, 178]. The maintenance dose of 100 µg is considered the gold standard in both adults and children, and must be increased to 200 µg [179] in unprotected patients (usually adults) and, according to some authors, in beekeepers [13]. Once the maintenance dose has been reached, the intervals between doses should be maintained at 4 weeks in the first year, and gradually increased up to 6-8 weeks in the following years, without any reduction in clinical
efficacy of VIT [180]. According to some authors [181-183] after the 3rd year of VIT the interval can be progressively lengthened up to 12 weeks. Other studies evaluated 6-month intervals; this extension is currently not recommended because it could affect the effectiveness of the treatment [182].

Although according to EMA pharmacovigilance data there are no reports of toxic effects of aluminum hydroxide in products for AIT, in VIT with a maintenance dose of 200 μg and in VIT with two different venoms, as a precautionary measure, it is preferable to use an aqueous extract for at least one of the two VITs [90]. A recent paper analyzed Aluminium (Al) concentration in urine and blood in two groups of patients: never treated with Al-depot SCIT vs Al-depot VIT treated. No differences were detected in urine Al-concentrations between the two groups, as well as in blood using free-gel monovetted. However, due to the small amount of the free-gel detections, data from blood remains inconclusive [184].

There are currently no guidelines in the literature on the management of product deficiency during maintenance, which occurred in 2016 due to the sudden unavailability of some extracts. A recent multicenter study prospectively collected data on VIT switching and reported that switching VIT from one manufacturer to another is a safe option to consider, if case of need, in patients who had previously tolerated VIT, even without reducing the previous maintenance dose, in a proper medical setting staffed by experienced personnel [185,186]. In patients who experienced previous severe SRs during VIT, the treatment should be restarted with a rush/ultrarush protocol in centres well experienced in HVA and VIT, or with a conventional protocol in less experienced centres.

Duration

In patients without specific risk factors VIT should be continued for 5 years [187]. Based on currently available literature, the recommended duration of VIT is 3-5 years in adults and
children [73,90,187]. In particular, one year of VIT did not provide sufficient protection in about a quarter of the treated patients [188].

After 3 years of VIT, 83% to 100% of patients remain protected against subsequent stings for 1 to 3 years after termination [158,159,188,189-193].

A VIT treatment equal to or longer than 5 years provides a more prolonged efficacy after the interruption [189,194,195]. At present there are no data on the maintenance of VIT protection for periods longer than 15 years, especially in the case of VIT with bee venom. Table 8 shows the risk factors for relapse after interruption of VIT.

When skin and serological tests turn negative, VIT can be safely interrupted. However, this rarely occurs [73]. After 5 years of VIT there is an average reduction of specific IgE compared to baseline of 58-70% overall, and a lower reduction in older patients or in patients with very severe onset reaction, not correlated with lower clinical efficacy [196]. Indeed, the decision of stopping VIT cannot be based solely on the reduction of specific serum IgE levels, since stung and protected patients during VIT show higher IgE levels at the end of the 5-year therapy, compared to not-stung patients, though clinically protected [196]. It has recently been shown that an increase in the IgG/IgE ratio correlates with the reduction of specific IgE and skin reactions in patients who have undergone at least 3-year VIT [197]. However, there is presently no validated test able to predict venom tolerance due to the variability of immunological parameters during VIT [198].

In clinical practice, the patient is often no longer stung during VIT, as he adopts rules of environmental prophylaxis. It becomes therefore difficult to decide whether or not to suspend VIT in the absence of field proof of protection. Even though sting challenge test is still the most reliable method and gold standard to monitor the effectiveness of VIT [90], its practice for the demonstration of VIT effectiveness cannot be implemented in some countries for critical ethical and management issues [199]. A micro-syringe challenge
method has recently been developed [200], whose validity awaits to be confirmed in further studies.

For the proper management of patients it is essential to know the risk factors that could negatively impact on VIT protection. According to the prevalent expert opinion, patients suffering from mast cell disease should be lifelong treated [201,202]. However, this suggestion is not confirmed by controlled studies [90]. According to a recent study on a selected population, mastocytosis should be considered in patients developing severe reactions at re-sting after VIT discontinuation. On this basis, patients with mastocytosis and HVA should be VIT-treated lifelong [203].

The decision to prolong VIT over 5 years should be shared with patients, based on specific risk factors and impact of quality of life; there is currently no contraindication to continue VIT over 5 years.

Patients should always be followed up over time, an aspect not properly considered by European and American guidelines. Based on current knowledge, the present panel of experts suggests:

- Patients not undergoing VIT but equipped with an adrenaline autoinjector because of a previous systemic reaction: follow up visit in case of re-sting and history collection at each re-order of adrenaline including a refresh in the training on device use. In the absence of re-sting, it is useful to schedule a follow up visit every 2 years, in order to perform skin and/or serological allergy tests before further adrenaline prescription.

- Patients undergoing VIT: skin reaction control and/or specific IgE determination at 3 and 5 years or in case of systemic reaction to field sting.

- Patients at risk of multiple stings or with risk factors for relapse after VIT interruption: follow up visit in case of re-sting and history collection at each re-order of adrenaline including a refresh in the training on device use.
Adverse reactions

Various reports in the literature reveal a high variation (0-46%) in the incidence of side effects due to VIT [204,205]. This is likely due to multiple factors, including different classification systems for severity, different quality of not purified, aqueous or depot extracts, different administration protocols.

A recent systematic review of the literature [90] examined 11 observational studies: VIT was associated with a 14.2% risk of adverse events in patients treated with bee venom and in 2.8% risk in those treated with vespids venom. Another systematic review [206] reported a mean frequency of 28.9% adverse events with bee venom, of which 50.4% were systemic reactions and 10% local extended reactions, respectively.

Large studies showed that the majority of reactions to VIT occurred in the build-up phase, including up to 20% systemic reactions (1.9% during build-up phase, 0.5% during maintenance on a total of 26601 injections), of which 8.4% moderate to severe [204].

LLRs to VIT are frequent, especially in the build-up phase; they do not represent a risk factor for subsequent systemic reactions, do not require a dose reduction and do not prevent reaching the protective dose. In the case of systemic reactions, it is preferable to reduce the dosage in the build-up phase, (e.g., by stepping down 1 or 2 doses) and continue with the last well-tolerated dose [90].

The following risk factors for systemic reactions during VIT have been identified: bee venom, high basal tryptase values in patients allergic to wasps, mast cell clonal diseases, rush and ultra-rush protocols [73,204,207,208].

However, some authors do not consider rush or ultra-rush induction protocols to be dangerous, since they were able to demonstrate a low risk of systemic reactions and safety profile equivalent or even better than slower protocols [163,209-211].
The use of depot extracts has been related to a lower frequency of local adverse reactions compared to the use of aqueous extracts [212,213]. A systematic review confirmed that the incidence of systemic reactions is significantly higher for bee venom than for vespids (25.1% vs 5.8%), while no differences were found regarding use of aqueous vs depot extracts in treated patients [214]. However, this review did not take into account the use of non-purified aqueous versus purified aqueous extracts. In fact, the use of purified aqueous extracts seems to correlate not only with a lower frequency of important local reactions, but also with systemic reactions, as compared to non-purified extracts [215,216].

In some double-blind, placebo-controlled studies, premedication with antihistamines improved VIT tolerance while maintaining efficacy [217-220]. On this basis, recent EAACI guidelines [90] recommend this medication, which can prevent extensive local reactions and mild systemic reactions. The possibility of masking warning signs and symptoms of more severe reactions, especially if rapid protocols are used, suggested the Italian expert panel to indicate this treatment as optional.

In patients developing systemic reactions to VIT and when premedication with antihistamines is not sufficient, off-label premedication with omalizumab [221,222] was successfully implemented.

**VIT and pregnancy**

Studies on the safety of VIT in pregnancy are limited [223,224], mainly for ethical reasons. One of the potential risks of immunotherapy, in addition to the management of possible adverse reactions, could be the induction of a Th2-Th1 cytokine shift, opposing the overall Th2 profile of pregnancy, which was claimed to avoid foetal rejection [225]. In a 1990 study, 26 patients with multiple pregnancies undergoing VIT were evaluated. The authors estimated a 3-5% risk of field sting anaphylaxis in women not undergoing VIT during
pregnancy, while the risk of anaphylactic reaction during VIT was 1% in the maintenance phase and 5% in the build-up phase. To this risk the possible severe consequences of anaphylaxis on the foetus should be added. The risk of maternal-foetal complications in pregnant women undergoing VIT was similar to that of women not undergoing VIT [224].

In a 2002 case report, a preterm delivery was reported due to placental abruption at week 24 in a woman who had started the build-up phase of immunotherapy during the first weeks of gestation. VIT was continued at the maintenance dose of 50 µg. Placental analysis demonstrated a Th1 pattern with infiltrated cytotoxic T lymphocytes [226]. In a more recent case report of in vitro fertilization, VIT was instead considered safe [227].

In conclusion, as confirmed by recent European Guidelines [228], immunotherapy should not be started during pregnancy. However, VIT should not be interrupted in case of pregnancy if patients are already undertaking and well tolerating VIT, considering the low risk of side effects [204].

VIT compliance

Compliance of specific immunotherapy is a serious problem in the management of patients with respiratory allergies [229]. In the case of VIT, a recent Italian study showed high percentages of compliance at 3 (95%) and 5 years (84%) of treatment [230].

Paediatric aspects

Although the efficacy of VIT is also known in children, there are no double blind, placebo-controlled trials in paediatric patients [231]. Treatment is recommended in children suffering systemic reactions with cardiovascular and/or respiratory involvement after hymenoptera stings [73,231].

In children with only cutaneous systemic reactions, VIT is not routinely performed
[90,232,233], as a long-term prospective study has shown that children with this type of reaction have a 10% risk of re-developing a systemic reaction [131]. However, there may be particular situations of increased risk of re-sting (e.g., children of beekeepers), possibly associated with parents’ and children’s concern or with distance from the emergency room, or unavailability of school staff administering antihistamine drugs. These conditions warrant VIT also in cases of urticaria alone [73].

As far as the risk of systemic reactions with respiratory or cardiovascular involvement in children is concerned, an observational study of paediatric patients followed for 15-20 years reported that the risk of re-developing anaphylaxis in untreated children was 32%, compared to 1-3% of those treated with VIT [154]. In a recent 6-years follow-up European paediatric study, 62% of children allergic to venom and not treated with VIT tolerated subsequent stings, whereas 18% had severe systemic reactions [234]. The proportion of therapeutic failure of VIT is lower in children than in adults (about 2% of treatments) [49,189,235].

Induction patterns in children are similar to those used in adults [131]. Regarding accelerated protocols, in a paediatric study [236] 43 children and adolescents (4 to 18 years old, with a 1-4 Muller grade systemic reaction after bee or wasp sting) undergoing ultrarush VIT were analysed; no systemic reaction was reported. A recent study confirmed the tolerability of the rush schedule in paediatric as compared to adult patients [237]. Another study evaluated the safety of rush 3-days induction protocols versus conventional 4-months schemes [238]: no differences were found between the compared protocols in terms of systemic reactions (19% and 23.2% with rush and conventional protocol, respectively). In another 2016 study [239] the ultra-rush Birnbaum’s induction protocol [240] (101 µg cumulative in 210 minutes) was compared in adults and children: 7.7% of systemic reactions in adults versus 3.7% in children. In general, it is important to underline that, as in
adults, it is preferable to avoid too rapid schedules when using bee venom [237].

Children have a better prognosis than adults with regard to the maintenance of efficacy upon discontinuation: in a Golden’s study with up to 20 years follow-up, only 5% of children with severe pre-treatment reaction re-developed a non-severe systemic reaction at re-sting [154], compared to 16% of adults [241].

Among 40 children treated with mean 3-year VIT, 50% developed a new anaphylactic reaction at a median follow-up of 13 years; notably, 95% had not received adequate follow-up after discontinuation of VIT [242].

In view of these data, the panel of experts suggests that VIT duration should be at least 5 years also in paediatric patients. Moreover, also in children periodic checks and suitable educational programs are necessary.

**MANAGEMENT OF THE PATIENT WITH CONCOMITANT DISEASES**

**Heart disease**

The presence of cardiovascular disease is an important risk factor in patients allergic to Hymenoptera venom due to severity of anaphylaxis after a sting. In fact, an increased density of mast cells has been identified in arterial intima and adventitia in ischemic cardiopathy, aortic valvular stenosis, hypertrophic cardiomyopathy [243]. Furthermore, mast cells in ischemic myocardium are richer in histamine and tryptase than the ones in healthy myocardium.

Venom components can induce the release of serotonin and adrenaline which increase platelet aggregation, with the chance of thrombosis being favoured by an increase in factor V and by the release of a thromboplastin-like substance from the wall of blood vessels. These and other substances released by mast cells would have a negative isotropic and
chronotropic effect. *De novo* synthesis during the anaphylactic reaction of LTC4 and PGD2 at cardiac level may result in vasoconstriction. Similarly, stimulation of H1 receptors in some patients with coronary artery disease may cause vasoconstriction of coronaries of large calibre, in contrast to the healthy myocardium [244]. The activation of the metalloproteinase also degrades the connective tissue of the atheromatous plaques, increasing ischemic risk. Physiologically, the decrease in blood pressure that occurs in the anaphylactic reaction leads to less perfusion of the sinus of Valsalvae and to coronary hypoperfusion.

Kounis syndrome [245], also called “cardiac anaphylaxis”, is characterized by the development of signs and symptoms that can be compared to those of a coronary syndrome. This syndrome may be due to the direct action of the venom on the coronary endothelium or to a degranulation of mast cells due to the allergic reaction with direct release of inflammatory mediators in the coronary vascular system (histamine, kinase, triptase) and synthesis of leukotrienes that act as powerful vasoconstrictor of the coronary arteries [246].

In patients allergic to Hymenoptera venom, in whom a subsequent allergic reaction may be more severe or even fatal, VIT has an elective indication even if there has been a myocardial infarction or a severe ventricular arrhythmia. In these patients, VIT was found associated to a low incidence of systemic reactions and to a certain efficacy [247].

The cardiopathic patient is often treated with beta-blockers and ACE inhibitors, which are common in the treatment of hypertension and heart failure. Beta-blockers can reduce the efficacy of adrenaline in the case of use to treat systemic reactions to Hymenoptera. However, their use is not contraindicated during VIT [228]. Indeed, very recently β-Blocker use did not seem to be clinically significant with respect to the need for adrenaline dosing among emergency department patients with anaphylaxis [248].

Their suspension, limited to the rash or ultra-rash induction phase of VIT, can be considered
and discussed with cardiologists. ACE inhibitors represent a risk factor for the severity of the reaction in patients not treated with VIT, however they do not seem to increase the risk of systemic reactions during VIT. According to a recent study [166] they would constitute a risk for reduced protection of VIT to insect challenge. Therefore, their suspension remains at the discretion of the clinician based on the risk/benefit ratio [16].

In conclusion, status of cardiovascular disease, its pharmacological treatment and the risk of anaphylaxis with consequent adrenaline administration should be carefully evaluated on an individual basis, preferably in concert with the consulting cardiologist, before starting VIT (Strength of recommendation D).

**Elderly patients**

According to European Academy of Allergy and Clinical Immunology (EAACI) and to American Academy of Allergy, Asthma and Immunology (AAAAI), VIT should be taken into consideration in older adults, even if they have experienced a non-severe systemic reaction, provided that they have risk factors such as: concomitant vascular diseases, treatment with ACE inhibitors and/or beta-blockers, severe COPD pictures, reduced quality of life due to the previous anaphylactic event [73,87].

To date, no data are available to demonstrate an increased risk of side effects or an increase in emergency treatments of this patients’ group (Strength of recommendation D).

**Malignancies**

Malignant neoplasias are considered absolute contraindications for specific immunotherapy with Aeroallergens, although not all guidelines agree. This contraindication has been established for safety and ethical reasons [249], since the risk of an exacerbation of
neoplastic disease by AIT is only theoretical, although a possible immunological interaction between neoplastic pathology, oncological treatments and AIT cannot be completely excluded. However, in patients allergic to Hymenoptera venom with a high risk of severe reactions to subsequent stings (e.g., previous life-threatening reaction or clonal mast cell diseases), VIT appears to prevent fatal events even in the presence of neoplasia. [228,250]. (Strength of recommendation D).

Autoimmune diseases and immunodeficiencies
Multi-organ autoimmune diseases in remission are considered by some guidelines relative contraindications for immunotherapy. If autoimmune diseases are clinically active, the contraindication is absolute [228]. (Strength of recommendation D).
VIT is not contraindicated in patients with organ-specific autoimmune diseases (e.g., diabetes mellitus, Hashimoto's thyroiditis, Crohn's disease, ulcerative colitis, rheumatoid arthritis), provided the disease is stabilized before starting treatment [251]. Strength of recommendation D.
As far as immunodeficiencies are concerned, they have a different impact and a different physio-pathological mechanism; also concomitant treatment with immunosuppressive drugs contraindicate AIT according to some guidelines since they could have a negative impact on the effectiveness of VIT. In particular, HIV infection is a relative contraindication to VIT that can be assessed on an individual basis. Strength of recommendation D. AIDS with a confirmed category C disease (according to CDC 1993 Atlanta Classification) is an absolute contraindication to VIT [228]. Strength of recommendation NR.

Mastocytosis
Anaphylaxis is the most severe clinical manifestation of systemic mastocitosis (SM) and
Hymenoptera stings are reported as the most frequent cause (19-53% of cases) [252].

The preferential association between mastocytosis and allergy to Hymenoptera venom is now well known and studied [100]. Prevalence of Hymenoptera venom allergy in the European adult population is between 0.3% and 8.9% and rises up to 20-30% in patients with mast cell disorders [3,252,152]. On the other end, prevalence of SM in the general population is 1-1.3 cases per 10,000 and is significantly higher in patients with Hymenoptera venom: 5-8% [25,252].

Patients with SM without skin involvement presenting Hymenoptera venom anaphylaxis are likely to represent a particular phenotype, with an excellent prognosis, predominant in male, with lower values of serum tryptase and lower proportions of bone marrow mast cells as compared to indolent forms. Moreover, this phenotype does not include other symptoms due to mediators release and affects mast cells and no other myeloid line [253]. In contrast, Hymenoptera anaphylaxis appears to be absent in patients with aggressive forms of SM, despite the greater “mast cell burden” [254]. In patients with onset of mastocytosis after hymenoptera anaphylaxis, progressions to aggressive forms or associated haematological malignancies are rarely described.

After an initial debate, mainly focused on the safety and efficacy profile of VIT in patients with mastocytosis [100], this treatment is now recognized as safe and efficacious [252,255,256], inducing protection from severe allergic reactions to subsequent stings.

Due to the reporting of life-threatening and even fatal reactions to Hymenoptera stings after discontinuation of treatment, long-term VIT may be recommended, probably with a lifelong duration [100, 203]. In patients not adequately protected by the usual maintenance dose of 100 μg, it is recommended to increase it to 200 μg [252]. Patients affected by SM with a history of anaphylaxis should always carry two adrenaline autoinjectors, a recommendation also valid for patients receiving VIT [252].
PROFESSIONAL ASPECTS

Hymenoptera stings are the most frequent cause of occupational anaphylaxis, attributable to a particular work environment [257].

Since exposure to repeated stings is one of the main key factors for the development of allergic reactions, persons working outdoors or in environments where Hymenoptera live are considered to be at high risk. In addition to the beekeepers [13], to whom a specific risk is recognized, other workers such as foresters, farmers and gardeners, truck drivers, masons, electricians [258,259] also show an increase in the incidence of systemic reactions; greenhouse workers are also exposed to bumblebee stings [8,260]. For these categories, Hymenoptera venom allergy can be considered a professional pathology [259,261] prompting the adoption of specific primary prevention measures [262]. Hymenoptera venom allergy is a recognized cause of work disability compelling to change or abandon the job for workers who have experienced an allergic reaction, in order to reduce the risk of exposure [263].

To allow the worker at risk to continue his activity, VIT is also recommended for moderate systemic reactions, given its degree of effectiveness [14,190,257,262].

Some European authors recommend verifying the efficacy of the treatment before the resumption of work through sting challenge [262], nevertheless this clinical practice being not allowed in Italian routine. A maintenance dose of 200 μg may be indicated for beekeepers [73]. Subjects with bumblebee occupational anaphylaxis have a low degree of cross-reactivity with bee venom and therefore should undergo VIT with bumblebee venom [8]. Since workers highly exposed to stings have a higher risk of relapse after VIT discontinuation, some experts recommend to continue the treatment until the profession risk is maintained [262].
A recent Italian study conducted on 184 patients with anaphylactic reactions to Hymenoptera venom showed an occupational cause in 17.4% of cases; among these, 71.8% continued to work, having been treated by VIT. Re-stung workers (31.2%) were effectively protected [261]. The impact of VIT on work activity is higher the greater is the occupational risk [263].

QUALITY OF LIFE

The history of previous allergic reactions to Hymenoptera has a negative influence on the quality of life of allergic subjects. Many of them are living everyday life in constant anxiety about being stung and possibly suffering the same or even more severe and potentially fatal reactions [264]. Questionnaires were validated to specifically evaluate the quality of life of subjects allergic to vespids [151,265], including Polistes dominula for patients in the Mediterranean area [266]. Randomized controlled clinical trials evaluating the impact of Hymenoptera venom allergy on quality of life confirmed that immunotherapy is associated with a significant improvement of the quality of life after one year from the beginning [151,267]. People undergoing immunotherapy have a better quality of life compared to those who are prescribed only adrenaline autoinjector, even if they suffered a systemic reaction of medium severity such as urticaria or angioedema. Moreover, sting challenge results in a significant improvement in disease-specific quality of life in patients allergic to Hymenoptera venom receiving VIT [265-268]. These findings should be taken into account when choosing whether to start immunotherapy in subjects who had a cutaneous systemic reaction, and in some cases immunotherapy should be preferred to the single prescription of adrenaline autoinjectors [81].
In case of children allergic to Hymenoptera, the disease can have an impact on the quality of life of their parents. Through specific questionnaires, it was shown that parents of Hymenoptera allergic children have a worse quality of life for the fear that their children can get stung and have severe consequences, in addition to feeling responsible for life and health of their children [269].

CONCLUSIONS
Allergic reactions to Hymenoptera stings can occur with varying degrees of severity and can sometimes be fatal. Although their epidemiological burden is similar to food allergy, the awareness of this problem is poor in the general population as well as among health care providers and political decision-makers. Similarly, the availability of acute emergency treatment (adrenaline autoinjector) and long-term immunotherapy, modifying the natural history of this allergy, is still poorly known. This appears paradoxical considering the innumerable scientific innovations on this subjects that appeared in literature over the past 5-10 years.

It is therefore mandatory to improve the knowledge and the management of this pathology, also considering that specific immunotherapy with venoms is currently the by far most effective form of allergen specific immunotherapy presently available.

This Consensus is a report accessible to healthcare professionals and to anyone looking for information on this pathology. In particular, this document can be used by specialists in daily clinical activity and can provide practical advice supported by scientific evidence on both diagnosis and therapy.

As in many other areas of medicine, there are aspects waiting for further studies in the coming years [90,151], to the advantage of allergic patients.
FUNDING

The authors declare that no funding was received for the present consensus.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTION

MB Bilò, V Pravettoni, P Bonadonna, M Mauro, D Bignardi, O Quercia, and E Novembre reviewed the literature and drafted the manuscript. All other authors critically reviewed the draft and approved the final manuscript.
REFERENCES


18. Biló MB. Anaphylaxis caused by Hymenoptera stings: from epidemiology to


45. Graif Y, Romano-Zelekha O, Livne I, Green MS, Shohat T. Increased rate and


63. Cousins L. Fetal oxygenation, assessment of fetal well-being, and obstetric


73. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U; EAACI Interest Group on Insect


allergy: a comparison study in field re-stings. World Allergy Organization J. 2018; 11:3-8.


114. Monsalve RI, Vega A, Marqués L, Miranda A, Fernández J, Soriano V, et al. Component-resolved diagnosis of vespid venom-allergic individuals: phospholipases and antigen 5s are necessary to identify Vespula or Polistes...


144. Cichocka-Jarosz E, Sanak M, Szczeklik A, Brzyski P, Pietrzyk JJ. Impact of


152. Niedoszytko M, de Monchy J, van Doormaal JJ, Jassem E, Oude Elberink JN. Mastocytosis and insect venom allergy: diagnosis, safety and efficacy of venom


161. Quercia O, Emiliani F, Pecora S, Burastero SE, Stefanini GF. Efficacy, safety, and


Treatment with American Polistes venom was ineffective in an Italian patient allergic to European Polistes. Allergy. 2007;62:966-7.


201. Oude Elberink JN, de Monchy JG, Kors JW, van Doormaal JJ, Dubois AE. Fatal anaphylaxis after a yellow jacket sting, despite venom immunotherapy, in two


contraindications to allergen immunotherapy: an EAACI position paper. Allergy. 2015;70:897-909.


246. Bach MK. Mediators of anaphylaxis and inflammation. Annu Rev. 1982;36:371-


251. Pfaar O, Bachert C, Bufe A, Buhl R, Ebner C, Eng P, et al. Guideline on allergen-specific immunotherapy in IgE-mediated allergic diseases: S2k Guideline of the German Society for Allergology and Clinical Immunology (DGAKI), the Society for Pediatric Allergy and Environmental Medicine (GPA), the Medical Association of German Allergologists (AeDA), the Austrian Society for Allergy and Immunology (OGAI), the Swiss Society for Allergy and Immunology (SGAI), the German Society of Dermatology (DDG), the German Society of Oto-Rhino-Laryngology, Head and Neck Surgery (DGHNO-KHC), the German Society of Pediatrics and Adolescent Medicine (DGKJ), the Society for Pediatric Pneumology (GPP), the German Respiratory Society (DGP), the German Association of ENT Surgeons (BV-HNO), the Professional Federation of Paediatricians and Youth Doctors (BVKJ), the Federal Association of Pulmonologists (BDP) and the German


259. Bonadonna P, Schiappoli M, Dama A, Olivieri M, Perbellini L, Senna G,


267. Dubois AEJ. Venom immunotherapy improves health-related quality of life in


### Table 1. IV adrenaline administration

<table>
<thead>
<tr>
<th>IV Bolus</th>
<th>1 ampoule diluted to 10 mL (100 µg/mL) → 0.5 mL (=50 µg) in bolus - or syringe pump 1 ampoule diluted to 50 mL (20 µg/mL) → 180 mL/hour for 1 minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV Infusion in syringe pump</td>
<td>1 ampoule of 1 mg, diluted to 50 mL with saline solution speed = 20 µg/mL</td>
</tr>
<tr>
<td>Infusion rate in syringe pump</td>
<td>5 µg/min 15 mL/hour 10 µg/min 30 mL/hour 15 µg/min 45 mL/hour</td>
</tr>
</tbody>
</table>
Table 2. Dopamine: hourly speed based on body weight and desired dosage.

<table>
<thead>
<tr>
<th>Kg pcs</th>
<th>5 µg/kg/min</th>
<th></th>
<th>10 µg/kg/min</th>
<th></th>
<th>15 µg/kg/min</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/min</td>
<td>ML/hour</td>
<td>µg/min</td>
<td>ML/hour</td>
<td>µg/min</td>
<td>mL/hour</td>
</tr>
<tr>
<td>40</td>
<td>200</td>
<td>1.50</td>
<td>400</td>
<td>3.00</td>
<td>600</td>
<td>4.50</td>
</tr>
<tr>
<td>50</td>
<td>250</td>
<td>1.87</td>
<td>500</td>
<td>3.75</td>
<td>750</td>
<td>5.62</td>
</tr>
<tr>
<td>60</td>
<td>300</td>
<td>2.25</td>
<td>600</td>
<td>4.50</td>
<td>900</td>
<td>6.75</td>
</tr>
<tr>
<td>70</td>
<td>350</td>
<td>2.62</td>
<td>700</td>
<td>5.25</td>
<td>1050</td>
<td>7.87</td>
</tr>
<tr>
<td>80</td>
<td>400</td>
<td>3.00</td>
<td>800</td>
<td>6.00</td>
<td>1200</td>
<td>9.00</td>
</tr>
<tr>
<td>90</td>
<td>450</td>
<td>3.37</td>
<td>900</td>
<td>6.67</td>
<td>1350</td>
<td>10.12</td>
</tr>
</tbody>
</table>

*200-mg ampoule: 2 ampoules in 5% glucose solution by 50 mL syringe pump (= 8000 µg/mL)*
Table 3. Doses of glucagon to administer during anaphylaxis

<table>
<thead>
<tr>
<th>Glucagon dosage</th>
<th>1-mg ampoule, diluted to 50 mL with saline solution = 0.02 mg/mL syringe pump infusion speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/hour</td>
<td>50 mL/hour</td>
</tr>
<tr>
<td>2 mg/hour</td>
<td>100 mL/hour</td>
</tr>
<tr>
<td>3 mg/hour</td>
<td>150 mL/hour</td>
</tr>
<tr>
<td>4 mg/hour</td>
<td>200 mL/hour</td>
</tr>
<tr>
<td>5 mg/hour</td>
<td>250 mL/hour</td>
</tr>
</tbody>
</table>
Table 4. Diagnostic criteria for the diagnosis of anaphylaxis [27]

<table>
<thead>
<tr>
<th>The three criteria for a likely diagnosis of anaphylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 For a naive patient</td>
</tr>
<tr>
<td>Acute onset (minutes or hours) of cutaneous end/mucosal symptoms (pruritus, flushing, lips-tongue-uvula swollen, hives and generalized urticaria)</td>
</tr>
<tr>
<td>Two or more of the following:</td>
</tr>
<tr>
<td>A. Cutaneous end/mucosal symptoms (pruritus, flushing, lips-tongue-uvula swollen, hives and generalized urticaria)</td>
</tr>
<tr>
<td>B. Respiratory symptoms (dyspnea, wheezing, bronchoconstriction, stridor, reduced peak expiratory flow, hypoxemia)</td>
</tr>
<tr>
<td>C. Decreased blood pressure and/or associated symptoms of the target organs (hypotonia, collapse, syncope incontinence)</td>
</tr>
<tr>
<td>D. Persistent gastrointestinal symptoms (abdominal pain, cramps, vomiting)</td>
</tr>
<tr>
<td>2 After exposure to a likely allergen</td>
</tr>
<tr>
<td>One or more of the following:</td>
</tr>
<tr>
<td>A. Respiratory symptoms (dyspnea, wheezing, bronchoconstriction, stridor, reduced peak expiratory flow, hypoxemia)</td>
</tr>
<tr>
<td>B. Decreased blood pressure and/or associated symptoms of the target organs (hypotonia, collapse, syncope incontinence)</td>
</tr>
<tr>
<td>3 After exposure to a patient’s known allergen</td>
</tr>
<tr>
<td>Reduction of blood pressure:</td>
</tr>
<tr>
<td>A. Infant and children:</td>
</tr>
<tr>
<td>&lt;70 mmHg from 1 mo to 1 yr</td>
</tr>
<tr>
<td>&lt;70 mmHg (+2 x yrs) from 1 to 10 yrs</td>
</tr>
<tr>
<td>&lt;90 mmHg from 11 to 17 yrs</td>
</tr>
<tr>
<td>B. Adults:</td>
</tr>
<tr>
<td>&lt;90 mmHg or a decrease &gt;30% from patient’s baseline values</td>
</tr>
</tbody>
</table>

These criteria allow the diagnosis of about 80% of the anaphylaxis as cutaneous symptoms are present in 80% of the anaphylactic reactions.

Table 5. Indications for performing diagnostic tests

Indicated in:
- Patients with a history of systemic reaction following hymenoptera stings

Not indicated in:
- Subjects with a positive family history for allergic reaction to Hymenoptera stings
- Subjects who have unjustified fear to develop a systemic reaction to Hymenoptera stings following news on media of fatal anaphylaxis
- As screening in the general population

Optional in:
- Patients with a history of large local reactions
Table 6. Allergologic diagnosis in Hymenoptera venom allergy: practical considerations

- Skin tests represent the gold standard of diagnostics, and should be performed at least 2 weeks after sting; if negative, they should be repeated after 1-2 months.
- Prick tests, even if positive, should be integrated with intradermal tests.
- A simultaneous testing of the same concentration of more venoms is to be preferred and only afterwards the next concentration should be tested.
- Skin tests with venoms are generally safe, even in patients with mastocytosis, if performed by trained personnel in a suitable environment.
- Validated methods are to be used for the determination of serum-specific IgE to venom allergens.
- There is no correlation between reaction severity and the scores of the diagnostic tests both in vivo and in vitro.
- The use of component resolved diagnosis (CRD) is indicated in case of poly-sensitization or negative allergy tests with a proven history of previous systemic reaction.
- Presently, CRD allows the diagnosis between allergy to *Apis mellifera* and *Vespula spp.* venoms; CRD value is limited in case of double positivity to Vespula-Polistes.
- The CAP-inhibition method is appropriate in case of double positivity to Vespula-Polistes, when CRD is not diriment.
- The Basophil Activation Test (BAT) should be carried out in highly specialized laboratories for diagnostic purposes, only in specific situations. Its role as a diagnostic tool in patients with mast cell disorders and negative venom-specific IgE and skin test results is still controversial.
- When a severe systemic reaction occur, baseline serum tryptase levels should be measured.
Table 7. Risk factors for reduced VIT effectiveness

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Adults are at higher risk than children, as subjects under the age of 16 generally have a more favourable prognosis. VIT in subjects under the age of 16 is not recommended for cutaneous/mucosal systemic reactions, except in case of high exposure risk or deterioration in quality of life [50,152,235,270].</td>
</tr>
<tr>
<td>Bee venom</td>
<td>The protection rate for bee venom is lower than the one for vespid venom [28,73,160,189,191,271]. The reason for this discrepancy is not yet fully understood. Recent studies of molecular allergy have shown that relevant allergens to bee venom may be poorly represented in some extracts for specific immunotherapy [107,272].</td>
</tr>
<tr>
<td>Severity of onset reaction</td>
<td>Patients with severe systemic reactions to Hymenoptera sting are less likely to have long-term protection (based on the number and frequency of stings received after suspension of immunotherapy) compared to patients with milder reactions [159,188,189,241,271,273].</td>
</tr>
<tr>
<td>Systemic reactions during VIT</td>
<td>Patients with adverse reactions to VIT are at greater risk of incomplete protection than patients who tolerate VIT [166,191,271].</td>
</tr>
<tr>
<td>High tryptase values and clonal mast cell diseases</td>
<td>In some studies, clonal mast cell diseases are correlated to lower clinical efficacy [207,274]. However, other studies do not support this conclusion and confirm a protection rate between 67% and 85% [100,152,255,256,275]. In a recent prospective study, protection after field sting in patients with clonal mast cell disorders was 86% [256]. Overall, VIT should be considered in these patients an effective and safe option.</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>A single work on 1532 patients reported that this category of drugs should be a risk factor for reduced clinical efficacy, demonstrated by “sting challenge” [94].</td>
</tr>
</tbody>
</table>
Table 8. High risk of relapse after VIT discontinuation in the following conditions:

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult population compared to paediatric population</td>
<td>[73]</td>
</tr>
<tr>
<td>Severe pre-VIT systemic reaction</td>
<td>From the data of 4 prospective studies recruiting 386 patients with severe reactions at re-sting, 4.1% had mild, and 14.5% severe pre-treatment reactions [187].</td>
</tr>
<tr>
<td>Allergy to bee venom</td>
<td>The risk of systemic reactions after discontinuation of VIT for bee was 16% compared to 8% of patients treated with wasp venom [189]. The reasons, not entirely known, are partly related to the amount of venom delivered with sting and to the amount of venom administered by VIT. It has also been hypothesized that in the extracts for VIT some important bee allergens may be missed or underrepresented [109].</td>
</tr>
<tr>
<td>Systemic reaction caused by VIT</td>
<td>Patients who developed systemic reactions to VIT had a 38% risk of re-sensitization compared to those who tolerated treatment (7%) [276].</td>
</tr>
<tr>
<td>Failure to achieve protection during VIT</td>
<td>[192]</td>
</tr>
<tr>
<td>Clonal mast cell diseases and elevated baseline tryptase levels</td>
<td>Please refer to the mastocytosis section.</td>
</tr>
<tr>
<td>Repeated stings</td>
<td>According to European studies, patients repeatedly stung after VIT discontinuation have a greater risk of systemic reactions that may become progressively more severe [189]. Some professions are particularly at risk such as beekeepers and gardeners who need an indefinite duration of treatment.</td>
</tr>
<tr>
<td>Persistence of high scores of diagnostic tests at 5-year VIT</td>
<td>[241]</td>
</tr>
</tbody>
</table>
Table 9. Paediatric aspects of Hymenoptera venom allergy

| Epidemiology | Hymenoptera venom allergy is the second cause of severe reactions in the paediatric age (20.2%), after food allergy [19].  
| Prevalence: asymptomatic sensitization: 3.7% [42] – large local reactions: between 0.9% [43] and 20.8% [44] - systemic reactions: <1% [15,42].  
| Risk factors for systemic reactions [45,46]: bronchial asthma, atopy (according to few studies)  
| Clinic: cutaneous and cardiocirculatory systems are mostly interested; skin symptoms are the only clinical manifestation in 60% of cases [47].  
| Prognosis: favourable regarding re-sting, both in studies with sting challenge [48] and field sting [49,50]. |

| Diagnostics | - The diagnosis is not different from that performed in adults  
| - The degree of skin sensitization does not correlate with the severity of the reaction [131]. |

| Specific Immunotherapy (VIT) | Indications:  
| - VIT is recommended in children developing a systemic reaction with cardiovascular and/or respiratory involvement [73,232].  
| - in children with only cutaneous systemic reactions, VIT is usually not carried out [90,233], due to the very low risk of developing more severe systemic reaction.  
| - children with increased exposure risk (e.g., children of bee-keepers) and/or children or their parents, who show anxiety, are living far away from emergency departments or attending school not staffed with personnel trained to administer antiallergic drugs, may undergo VIT also in cases of skin reactions only [73].  
| Risk of anaphylaxis at re-sting:  
| - children not undergoing VIT: 32% vs 1-3% of treated with VIT [154].  
| - recent European study with 6-year follow-up: 18% of non-VIT treated (while 62% tolerated subsequent stings) [233].  
| Efficacy:  
| - the percentage of therapeutic failure of VIT in children is lower than the one of adults (about 2%)[49,189,235].  
| - the prognosis after VIT discontinuation is better than that of adults: only 5% of children with severe pre-treatment reaction develop not severe systemic reactions at re-sting [149], compared to 16% of adults [241].  
| Induction schemes:  
| - as in adults [131].  
| - accelerated (rush, ultrarush) and clustered protocols are well tolerated by children [236-240]  
| - as in adults, avoid excessively rapid patterns with bee venom  
| - as in adults, the maintenance dose is 100 mcg, to be increased to 200 mcg in unprotected patients  
| Duration:  
| - the panel of experts suggests at least 5 years of VIT in the paediatric age  
| - proper follow up and appropriate educational programs are necessary. |

| Quality of Life | - The allergy to hymenoptera venom in children can have a negative impact on the quality of life of their parents.  
| - There are specific questionnaires on the quality of life for paediatric patients allergic to Hymenoptera and for their parents [269]. |

| Use of adrenaline | Dose: the use of i.m. adrenaline in the vastus lateralis muscle is recommended, at a dosage of 0.01 mg/kg (maximum dosage 0.3 mg)  
| Autoinjector:  
| - the fixed dosage involves a risk of administering a higher or lower dose of adrenaline; for children weighing between 15 and 30 kg and severe anaphylactic reaction or concomitant bronchial asthma, it is advisable to use the injector with adult dosage.  
| - it should be prescribed to children with systemic reactions (not just cutaneous reactions), with a high exposure risk, risk factors for lacking clinical protection, elevated baseline mast cell tryptase levels or mast cell disorders. [28,33,70,71] |
Box 1: Levels of evidence and grade of recommendations [2]

<table>
<thead>
<tr>
<th>Level of evidence</th>
<th>Grade of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level I</td>
<td>Systematic reviews, meta-analysis, randomized control trials</td>
</tr>
<tr>
<td>Level II</td>
<td>Two groups, nonrandomized studies (e.g., cohort, case–control)</td>
</tr>
<tr>
<td>Level III</td>
<td>One-group nonrandomized (e.g., before and after, pretest and posttest)</td>
</tr>
<tr>
<td>Level IV</td>
<td>Descriptive studies that include analysis of outcomes (single-subject design, case series)</td>
</tr>
<tr>
<td>Level V</td>
<td>Case reports and expert opinion that include narrative literature, reviews, and consensus statements</td>
</tr>
</tbody>
</table>

**Grades of recommendation**

| Grade A | Consistent level I studies |
| Grade B | Consistent level II or III studies or extrapolations from level I studies |
| Grade C | Level IV studies or extrapolations from level II or III studies |
| Grade D | Level V evidence or troublingly inconsistent or inconclusive studies at any level |