Diagnosis of hymenoptera venom allergy – with special emphasis on honeybee (Apis mellifera) venom allergy

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▌ Abstract

Hymenoptera stings occur very frequently and usually are not dangerous. The normal reaction after sting includes such symptoms as: mild swelling, redness and pain lasting from several minutes to several hours. In contrast, in people with allergy to insect venom, severe local reactions and systemic symptoms, including anaphylactic shock, may occur. Diagnostic tests should be performed in persons who experience systemic symptoms after a sting. The basic tests used in the diagnosis of allergy to hymenoptera venom are skin tests and detection of venom-specific IgE antibodies. If it is not possible to make a diagnosis on the basis of these data, specialized diagnostic tests, such as cellular tests, inhibition test and determination of tryptase and carboxypeptidase, can be performed. The presented study provides an overview of currently used diagnostic methods, both those used in everyday practice and allergy tests, which are available only in selected specialized centres.

▌ Key words

hymenoptera, allergy, honeybee venom, diagnostic tests

INTRODUCTION

Hymenoptera stings occur very frequently, however, usually they are not dangerous. In a healthy (i.e. non-allergic) person the reaction after the sting is limited to the occurrence of minor local symptoms. In contrast, in people with allergy to insect venom, severe local reactions and systemic symptoms including anaphylactic shock may occur.

Allergy to hymenoptera venom occurs in people of any age; however it is more common in adults than in children. Allergic systemic reactions occur in 0.3–7.5% of the population [1, 2, 3, 4, 5, 6, 7, 8], and in children they develop in less than 1% of the population [4], while severe local reactions are observed in 2.4–26.4% of people [2, 3] and in up to 19% children [4]. In Poland and Central Europe, allergic reactions after hymenoptera sting are mainly caused by honeybees (*Apis mellifera*) and wasps (*Vespula vulgaris, Vespula germanica*), and rarely by other hymenoptera insects, such as: hornets (*Vespa crabro*) and bumblebees (*Bombus spp.*).

In person who experience systemic symptoms after a sting diagnostic tests should be performed. These tests are the basis of further management and, possibly, treatment [9]. To date, no universal diagnostic test of high sensitivity and specificity has been introduced which could be routinely used in practice and would allow predicting the risk of severe allergic reactions after a sting. In many patients it is necessary to perform several tests which, combined with a history, allow the physician to diagnose allergy and decide on implementation of an appropriate immunotherapy. This article provides an overview of currently used diagnostic methods, both those used in everyday practice as well as allergy tests, which are available only in selected specialized centers.

MEDICAL HISTORY

Identification of insect. Because the patient may have problems with identification of the insect responsible for the sting, the physician has to be familiar with the basic differences in the appearance of insects, he/she should know which insects leave a sting in the skin, and the conditions which encourage insects to attack the victim.

- **Honey bee** (*Apis mellifera*): characterized by a nearly uniform dark brown colour. After the sting, the entire venom apparatus with venom reservoir remains in the skin; therefore, it is very important to remove it from the skin as soon as possible. Bee stings are usually experienced near apiaries and orchards.
- **Wasps** (*Vespidae*): have a black – yellow or dark brown – yellow colour. The genus *Vespula* usually build their nests in the ground. In contrast, the nests of the genus *Dolichovespula* are attached to branches of trees or in arbors and attics. Wasps do not leave the venom apparatus in the skin after stinging. The most common stings are associated with consumption of sweets and drinks, especially in the open air. This is due to the fact that wasps feed on the sugars and are attracted by the sweet food.
- **Hornets** (*Vespa*): evoke awe because of their considerable size which can reach 35 mm. Hornets build nests in hollows, attics and arbors. They also do not leave a sting in the skin of the victim.
- **Bumble bees** (*Bombus spp*): characterized by a stocky physique, abundant feathering, and yellow, orange or white

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stripes on the thorax and abdomen. They fly slowly near the ground with a loud noise. They are very calm insects which sting in extremely rare situations, such as on destruction of their nests.

Symptoms after insect sting. The normal reaction after sting includes symptoms such as: mild swelling, redness and pain lasting from several minutes to several hours. These symptoms may sometimes be more severe if the sting involves areas such as mucosa of the mouth, or areas which are rich in loose connective tissue, such as eyelids, lips or fingertips.
- Extensive local reactions result from an allergic reaction and are defined as swelling with a diameter exceeding 10 cm which lasts longer than 24 hours. These signs are usually accompanied by erythema, oedema, local itching and pain [10, 11].
- General symptoms of an allergic reaction after the sting may involve:
  ○ skin: urticaria, itching and angioedema;
  ○ respiratory system: shortness of breath and wheezing (due to laryngeal oedema or bronchospasm);
  ○ digestive tract: nausea, vomiting, diarrhea and abdominal pain;
  ○ cardiovascular system: hypotension, light-headedness, loss of consciousness;
  ○ central nervous system: headache and dizziness [12].

In order to assess severity of systemic reactions, Mueller's classification is used, in which 4 degrees of severity of an anaphylactic reaction can be distinguished, ranging from SYS I (mild) to SYS IV (severe).

Risk factors. In order to complete a medical history the patient should be asked for his/her general health, with emphasis on cardiovascular disease and atopy, medication and social status (nature of work, place of residence, and the way of spending free time).

Factors which increase the risk of stings:
- beekeepers and their families;
- living near an apiary;
- jobs such as fruit vendor, baker, confectioner, firefighter, farmer;
- spending time outdoors.

Factors which increase the risk of anaphylactic reaction:
- history of severe allergic reaction (grade III or IV, severe bronchospasm) [13];
- age (persons aged over 40);
- cardiovascular disease;
- asthma;
- taking certain medications (beta blockers, including eye drops, ACE inhibitors, NSAIDs);
- physical and mental fatigue;
- increased levels of tryptase;
- mastocytosis.

DIAGNOSTIC TESTS

In patients with general signs/symptoms after a sting, additional diagnostic tests must be performed [12, 13]. Additional diagnosis is not recommended in patients without general allergic reaction, because the positive results are found in 20–30% of the adult population with a relatively low

probability of systemic reactions (17%) after the subsequent sting [7, 16].

Currently, the basic tests used in the diagnosis of allergy to hymenoptera venom are skin tests and detection of venom-specific IgE antibodies. In practice, both of these tests are carried out, which in conjunction with the medical history, allow to approximately predict the risk of systemic reaction after the subsequent sting. If it is not possible to make a diagnosis on the basis of these data, specialized diagnostic tests, such as cellular tests, inhibition test and determination of tryptase and carboxypeptidase, can be performed.

Skin tests. Skin tests may be performed in 2 ways: as the prick tests or intradermally. Prick tests start with venom concentration of 0.01 µg/ml and the result is read after 15 minutes. If the result is negative, the concentration of venom is increased 10 times. The tests are stopped when a positive result is read, or when a venom concentration of 100 µg/ml is achieved. Increasing concentrations should be applied every 30 minutes. In the case of negative prick tests, it is recommended to perform intradermal tests. In the intradermal tests, increasing venom concentrations (from 0.001 µg/ml – 1.0 µg/ml) are used [13, 14, 16, 17]. The positive skin tests are obtained in 70–90% of all individuals who experienced the sting reaction [17, 18]. It should be noted that skin tests may be negative in up to 50% of patients in the first weeks after the sting [19]. Therefore, they should be performed not earlier than 3–6 weeks after the allergic reaction to the sting [12, 19]. When the result of skin test is negative, it can be repeated after 6–12 weeks [18, 20, 21].

***In vitro* assay.** *In vitro* assays are another method used for the diagnosis of allergy to hymenoptera venom and rely on the determination of venom-specific IgE antibodies. These tests are conducted in all patients with negative results of skin tests and in case of active skin disease or taking medications reducing skin reactivity (for example tricyclic antidepressants), which represent contraindication for performing skin tests [20, 21]. However, in 15–20% of patients, despite the positive history and positive skin tests, the results of *in vitro* tests are negative. Moreover, results of these tests do not correlate with the severity of clinical symptoms, which is emphasized in literature of the subject [7, 16, 22, 23].

The level of specific IgE may be very low or undetectable in the first days after the sting and gradually increases during the following days and weeks. After this initial phase, the IgE concentration decreases slowly with individually variable rates. Concentration of specific IgE is usually analyzed 4–6 weeks after the sting. Some authors suggest performing these determinations after 2 weeks. However, if the results are negative then they should be repeated after the subsequent 2–4 weeks [24, 25].

Currently, there are several tests based on the CAP or older (RAST) technique. The CAP technique is an example of enzyme-linked immunosorbent assay (ELISA). Using the CAP tests it is possible to determine specific antibodies not only to the 'whole' venom, but also to its individual components, such as phospholipase A$_2$ (Api m 1), which is the major allergen of honeybee venom [24]. It is known that in 97% of patients with bee venom-specific IgE antibodies, IgE antibodies to Api m 1 are also detected [26]. In addition, IgE antibodies to Api m 2 (hyaluronidase), Api

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m3 (acid phosphatase) and Api m4 (melittin) were present in respectively 51%, 60% and 31% of patients [27].

An example of a method based on the CAP is the ImmunoCAP test (Phadia) [26] in which a recombinant form of Api m1 is used. In this assay, the selected antigen (in this case – recombinant form of Api m 1) is covalently coupled to a solid phase (3D cellulose polymer). The antigen reacts with specific anti-Api m1 IgE present in the patient's serum. After washing off free IgE, the enzyme labeled antibodies against specific IgE are added and incubated. Then, unbound enzyme-anti-IgE are eluted and the bound complex is incubated again with a suitable substrate of the enzyme. The product of the enzymatic reaction exhibits fluorescence. After stopping the reaction, the fluorescence of the eluate is measured, which is directly related to the amount of specific IgE.

In the RAST technique, the anti-IgE antibody used in the reaction is isotopically labeled. These antibodies bind to specific IgE present in the analyzed serum. At the end of the protocol the radioactivity of the eluate is measured.

It should be emphasized that these tests are currently based on recombinant allergens, which significantly increases their specificity. It was shown that 15% of patients without a history of allergic reaction exhibited the allergic reaction to bee venom, while none of them responded to recombinant venom allergens (phospholipase A_2, hyaluronidase) used separately or together in a panel with melittin [29].

In the case of negative results in both skin tests and specific IgE determinations it is advisable to repeat these diagnostic tests after 3–6 months. It was noted that the highest concentration of specific IgE develops 6 months after the allergic reaction to a sting and can be detected in 96% of patients with allergy to bee venom [30].

Cellular tests. If double-performed routine tests fail to detect allergen-specific IgE, the IgE independent response may be suspected. In this case, an alternative diagnostic method involves a histamine release test or cellular allergosorbent test (CAST) [13, 16] and basophil activation test (BAT). These tests, known as cellular tests, are also used in cases of divergent results of skin tests and specific IgE measurements. An important aspect is that they are very sensitive and specific tests [31]. However, they are not commonly used in practice [30].

Histamine and sulfidoleukotriene release tests. Using the RAST or ELISA methods, levels of cellular mediators such as histamine and sulfidoleukotrienes can be measured. These substances are released by basophils after their binding of IgE antibodies (particularly after addition of cytokines such as IL-3). It was found that the sulfidoleukotrienes release test is more sensitive than the test involving histamine release [32].

The standardized and commercially used test of sulfidoleukotriene C4 release is termed CAST (cellular allergosorbent test) and is based on ELISA [33]. Sensitivity of this test was 88.5% and 94% for wasp and bee venom, respectively (patients with a positive history were studied) [32].

Basophil activation test – BAT. Basophil activation test is based on flow cytometry, which allows analysis of the cells in their natural environment, i.e. in the whole blood sample without any additives, which could affect the activity of basophils and influence the results of analysis. Using flow cytometry it was found that basophils which are *in vitro* stimulated with allergen can change their immunophenotype. This is manifested by increase in expression (upregulation) of specific proteins (markers) on basophils surface. There are 2 types of proteins which are important in the diagnosis of allergy to hymenoptera venom – CD63 and CD203c. Basophils at rest show on their surfaces moderate expression of CD203c and very low expression of CD63, while inside the cell very high levels of these markers are found. In response to a specific allergen, anti-IgE or other stimulant basophils show a typical for them activation pattern. Initially, rapid increase in expression of CD203c is observed, which may be accompanied by an increased expression of CD63. Then, basophil's strong expression of CD63 persists (or not) without concomitant expression of CD203c. BAT is based on measurements of concentrations manifested by CD63 and CD203c after incubation with venom at various concentrations [34].

Sensitivity of BAT ranges from 77 – 100% and its specificity varies from 70 – 100%, as demonstrated in numerous studies [32]. The most pronounced diagnostic value of BAT is achieved when it is conducted together with skin tests and specific IgE determination. BAT allows identification of the insect responsible for the allergic reaction in most patients in cases of negative skin tests and negative specific IgE results or discrepancies in these tests. In addition, some studies have documented the usefulness of this test in the monitoring of immunotherapy results.

Cross-reactions and inhibition test. In 30 – 40% of allergic patients from central Europe, allergy is shared for bee and wasp venom [24, 35]. However, the respective data vary considerably depending on the diagnostic methods used. In the case of *in vitro* methods the overlap reaches up to 64%, for skin tests – 48% and for BAT – 17% of the allergy cases [35]. The double sensitization (DS) may be due to 3 causes which occur individually or in combination:
- independent sensitization to both venoms (rare);
- cross-reactions between bee venom hyaluronidase (Api m2) and wasp venom hyaluronidase (Ves v2), or between dipeptidyl peptidase of bee venom (Api m5) and dipeptidyl peptidase of wasp venom (Ves v3);
- cross-reactions with carbohydrate epitopes CCDs (cross-reactive carbohydrate determinants) [24, 36].

Cross-reactions are due to the fact that specific IgE antibodies can 'recognize' similar epitopes of different allergens. The main allergens of hymenoptera venoms show many similarities in their structure. The structure of bee venom hyaluronidase in 50% is identical to wasp venom hyaluronidase. This enzyme is responsible for the cross-reactions in the case of bee and wasp venom allergy. The other example is bee venom phospholipase A_2, which is in 53% identical to phospholipase A_2 from bumblebee venom [23]. The cross-reactions associated with hyaluronidase seldom reflect similarity in the sequence of the peptide residues. Much more often the cross-reactions are caused by CCDs [35]. Both hyaluronidase, phospholipase A_1 and phospholipase A_2 are glycoproteins rich in CCDs and, therefore, they may induce the formation of anti-CCDs IgE after insect sting. It should be added that cross-reactions can be also caused by CCDs from other sources. Allergens, in fact, are very widespread; for example, they are present in pollen of grasses or in some food products, such as molluscs [24].

In a patient who exhibits a double sensitization to honeybee and wasp venom, an additional test (inhibition test) may be performed. Using this test it is possible to distinguish whether in fact the reaction involves allergy to both venoms or positive results are due to cross-reaction of IgE antibodies [13]. The inhibition test can be performed using the RAST or Western blotting after modifying them by addition of the initial stage [35, 37]. In the initial stage, called the inhibition phase, the patient's serum is incubated separately with both venoms or only with the relevant epitopes of these allergens. As a competitive inhibitor of CCDs binding with anti-CCDs IgE, bromelain is used (a glycoprotein rich in the CCDs). Depending on the results of inhibition test the following therapeutic approaches are recommended:

- lack of mutual inhibition – if insect, which caused anaphylaxis is unknown, it is necessary to conduct immunotherapy using both venoms;
- partial or variable inhibition – if insect, which caused anaphylaxis is unknown, it is necessary to conduct immunotherapy using both venoms;
- unilateral total inhibition – it is advised to conduct immunotherapy using venom which caused inhibition.

The future perspectives in solving the problems of cross-reactions involve recombinant allergens, which are free of CCDs. Diagnostic tests based on recombinant allergens are characterized by a significantly higher specificity, compared to the allergens isolated directly from insect venoms. This helps to clearly identify allergy to the relevant venom.

IgG4 antibodies. Another test used in the diagnosis of allergy to bee venom is determination of specific IgG4 antibodies, the concentration of which reflects the level of exposure to stings [22]. The protective role of these allergen-specific antibodies in beekeepers is emphasized [38]. Their concentration correlates with the number of stings and duration of work in the apiary [23, 38, 39, 40]. Furthermore, an increased level of IgG4 is observed following specific immunotherapy, even if neither the concentration nor the ratio of specific IgE/IgG correlate with clinical response to immunotherapy [23, 39, 40]. The increased IgG4 level has also been shown to manifest no relation with the clinical symptoms after the sting. Therefore, in routine diagnosis, determination of IgG4 concentration is not recommended.

Serum tryptase. In assessment of a severe allergic reaction risk after subsequent stings it is recommended to determine serum level of tryptase [41, 42, 43, 44]. Recent studies have shown that elevated levels of serum tryptase are associated with a very high risk of severe anaphylaxis following a subsequent sting [13, 22, 23, 45]. According to the latest guidelines, it is recommended to determine serum tryptase in patients with severe anaphylactic reaction after the sting [41]. In the available literature, no information is available on concentrations of tryptase in beekeepers' sera.

As tryptase is an enzyme present in mast cell granules and in much smaller quantities (about 500 times less) in basophils, and the test involves assessment of mast cells stimulation [46]. In the course of mast cell degranulation, tryptase is secreted simultaneously with histamine, but its diffusion into the tissues is much slower than that of histamine; the highest levels of tryptase in the serum are reached between 60 – 120 minute following the start of an anaphylactic reaction. Therefore, its concentration should be measured 15 minutes to 3 hours after the beginning of the allergic reaction. Nevertheless, some authors report that determination of baseline serum tryptase, even before an allergic reaction or at least 24 hours after it, helps in defining whether it in fact involves anaphylaxis [37]. Measurements of serum tryptase are usually conducted applying CAP [28]. In a healthy person without anaphylactic reaction in the recent few hours, serum tryptase levels are undetectable (<1 ng/ml). In patients with anaphylaxis, however, tryptase concentration may be increased slightly above the norm or values above 100 ng/ml can be reached. Currently, it is assumed that the normal concentrations of tryptase range from 1 – 11.4 ng/ml [34, 37]. An important aspect of such measurement involves the fact that tryptase concentrations manifest a significant correlation with severity of clinical symptoms [22], especially with a decrease in blood pressure in the main arteries [44, 47]. In addition, in patients with mastocytosis (values above 20 ng/ml [46]), acute myeloid leukemia, myelodysplastic syndrome or end-stage renal failure, elevated levels of this enzyme are observed [49, 50, 51]. A positive relationship has been demonstrated between the levels of tryptase and age, and gender-dependent differences in its concentrations documented. Concentration of serum tryptase is higher in the elderly [51]. In women it is about 0.2 ng/ml, which is higher than in men. However, the concentration showed no correlation with atopy [22].

Tryptase is produced in mast cells in the immature form of 2 isoenzymes (α – protryptase and β – protryptase), which are transformed into mature forms (α – tryptase and β – tryptase). The α form is only secreted constitutively, whereas the β form is primarily secreted during mast cell degranulation. Thus, the mature form of β – tryptase is responsible for the increase in total tryptase levels during anaphylaxis. Using commercial tests it is possible to determine total tryptase (mature and immature α and β forms) or only mature α and β forms of this enzyme [52]. In order to distinguish mastocytosis form anaphylaxis, α and β isoforms of tryptase should be determined separately. Patients with mastocytosis exhibit high baseline levels of both isoforms, while patients after an anaphylactic reaction have normal baseline levels of α-tryptase. The concentration ratio of total tryptase (α and β) to β-tryptase ≤ 10 indicates the anaphylactic reaction, and the ratio of ≥ 20 is typical for mastocytosis.

Elevated levels of serum tryptase are characteristic for anaphylaxis caused by insect sting or a parenterally given drug, when a decrease in blood pressure has occurred during allergic reaction. However, in the case of anaphylaxis induced by allergen given by the oral route (usually food) or anaphylactic reaction not paralleled by a decrease in blood pressure, levels of tryptase may be normal [52].

Carboxypeptidase A3 – CPA3. Recent studies on new markers of anaphylaxis have demonstrated the usefulness of serum carboxypeptidase A3 (CPA3) determinations. CPA3, like histamine and tryptase, represents another mediator released during mast cells degranulation, caused by anaphylaxis [53]. In patients with clinical signs of anaphylaxis, serum concentration of carboxypeptidase A3 was found to exceed 14 ng/ml. In addition, the elevated levels of CPA3 persist longer than an increased concentration of tryptase, and levels of these two markers fail to correlate with each other. In patients who developed clinical signs of anaphylaxis but manifested a normal serum tryptase level, the concentration of carboxypeptidase A3 was found to be elevated [37].

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Thus, most useful might be the determination of the entire panel of mast cell mediators, including histamine, tryptase, carboxypeptidase A3, chymase, PAF, and others. However, such a suggestion requires confirmation in further clinical trials.

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