Appendix 1: Scientific research that has studied a prognostic factor that would reliably show more severe systemic allergic reactions (anaphylaxis), or milder or major local allergic reactions after a wasp, bee or hornet sting.

Author, year of publication	The objective of the study	Research epidemiological basis	Methods of data collection	Observed population/ environment/period	Results	Main findings
Parker et al. [1982] (23)	Identify risk factors for a systemic reaction using an insect sting provocation test.	Prospective observational cohort study.	A provocation test was performed on 21 patients, 5 of whom had a history of a large local reaction after a previous sting, 16 of whom had a history of a systemic reaction to the sting. No one received immunotherapy. Just before the provocation test (bees, wasps, hornets), skin tests were performed and sIgE and sIgG antibodies were measured. • IgE: • positives were expressed as the percentage of sIgE-bound serum of the non-sensitized person (negative control) and the levels, that were higher than 200%, were evaluated as positive If several provocative tests were performed on one patient (with different insects (wasp and bee), the next was performed at least 60 minutes after the first.	21 patients (10 women, 11 men). Mayo, United States of America. From June 1979 until October 1980.	21 subjects, 5 with a history of LLR and 16 with a history of SR at the previous sting. In the LLR group, 7/21 skin tests were positive before the provocation test, and in the SR group, 30/34 skin tests were positive. sIgEs were at 63% to 789% for LR and at 66% to 801% for SR. sIgG antibodies ranged from <5 to 332 U/ml, levels were similar in both groups. 55 provocation tests (bee stings 14, wasps 11, hornets 8): 7 systemic reactions were induced, which were only in the SR history group. The article describes each patient individually with a systemic reaction, but does not provide an assessment of the systemic reaction compared to the previous – first – systemic reaction. There is also no description of factors (sex, age, other factors) that would predict a recurrent systemic response (small observed population). For LLR, 1/5 of the patients had a negative skin test and negative sIgE; 1/5 of patients had a positive skin test and positive sIgE; 2/5 had a negative skin test and positive sIgE; and 1/5 positive skin test and negative sIgE. For SR, 4/16 patients had a positive skin test and negative sIgE; 0/16 a negative skin test and positive sIgE; 1/16 a negative skin test and negative SIgE; and 11/16 a positive skin test and positive sIgE. 7 individuals (3 in the LR history group and 4 in the SR history group) who tolerated the first provocation test repeated it with venom a total of 27 more times. Of these 7, skin tests were now positive at 10/15 in the LLR group and at 10/12 in the SR group. sIgE levels ranged from 103% to 737% for the LR group and 85% to 2389% for the SR group before the second provocation test. IgG antibodies were the same in both groups. All other provocation tests (27 in total) were without a systemic response.	There is no *in-vitro* or *in-vivo* test that can predict with certainty the clinical immune status of insect stings for sensitized patients. It is still the most reliable method for referral to immunotherapy provocation test.
Blaauw et al. [1984] (24)	To present experience with diagnostic tests to identify allergy to insect venoms.	Prospective observational cohort study.	106 patients underwent an insect sting provocation test: skin tests, sIgE, sIgG level, and sIgG / sIgE ratio were measured. 86 patients with a positive history, skin tests and sIgE were eligible for immunotherapy according to the defined criteria. The severity of SR was assessed according to the Müller's grading system.	106 patients. Helmond, Netherlands. From May 1979 until August 1983.	86 patients had a history of SR and a positive skin test and sIgE for insect venom. 29 of these patients responded with a systemic response and were candidates for immunotherapy. Of the 29, 39% responded to wasp venom provocation and 61% to bee venom provocation. This is due to the fact that 80% of the patients in the group of 86 patients were beekeepers. The article does not describe whether the reactions after the provocation test were more severe or lesser with respect to the Müller's grading system, nor other risk factors. A comparison of diagnostic data from 29 responders and 57 non-responders from 86 patients showed that a provocative test could provide evidence for an insect sting allergy that manifests itself with a severe systemic reaction and requires immunotherapy. 57 patients without a reaction had a provocation test repeated after six months to see if the allergy increased over time. No one reacted with serious symptoms. In the group of the other 20 patients, 2 responded with SR, including one who had negative sIgE and one with negative skin tests. Both patients responded to the provocation test with a more severe systemic response than after the first sting (grade IV).	The study defined the importance of provocative tests in assessing a severe systemic reaction after an insect sting.
Kampelmacher et al. [1987] (25)	Clarify doubts about an insect sting provocation test that clinical data, sIgE and skin tests, and a positive medical history for SR are reliable tools in diagnosing and deciding on immunotherapy.	Prospective observational cohort study	90 patients (51 males, 39 females) with previous SR for bee stings (15) or wasp stings (74) or both (1) were admitted to the hospital intensive care unit for a provocation test with a resting. The severity of SR was assessed according to the Müller's grading system. 20 of them had a confirmed allergic disease (allergic rhinitis, food allergy, drug allergy).	90 patients with a previous systemic reaction. Utrecht, Netherlands. Summer of 1983 and 1984.	Of the 90 patients who underwent the provocation test, 25 (28%) responded with recurrent SR, of which 5 (20%) had allergic disease (allergic rhinitis, food allergy, drug allergy). 65 (72%) responded to the provocation test with a large local reaction only, of which 15 (23%) patients had allergic disease (allergic rhinitis, food allergy, drug allergy). A comparison of the assessment of the severity of the systemic reaction before and after the provocation test showed: • lower severity in 80 patients, • increased severity in 2 patients, • same severity in 8 patients. They did not demonstrate a statistically significant association between the severity of the first reaction and the severity after the provocation test. The interval between the last SR and the provocation test (> 1 year and <1 year) did not show a statistically significant difference between SR and non-responders. There was no statistically significant difference in age, sex, and the presence of other allergic diseases among patients who did not respond to the provocation test. However, a negative skin test and/or sIgE does not preclude a recurrent systemic reaction.	The long-lasting value of the provocation test has proven to be good as the results of the repeated provocation test were the same. Skin tests and sIgE are useful as diagnostic tools in determining the insect in question. The provocation test provides information on the probability of a recurrent systemic reaction.

Author, year of publication	The objective of the study	Research epidemiological basis	Methods of data collection	Observed population/ environment/period	Results	Main findings
Pucci et al. [1994] (26)	To determine whether a short interval (<2 months) between two stings affects the development of allergies (SR) for insect stings.	A retrospective observational cohort study.	The study compared the time interval between repeated insect stings in 120 allergic patients who experienced the first insect sting with no systemic reaction (73 for wasps and 47 for bees). They were interested in whether the short interval between the first and resting was a predictive factor for the severity of the systemic response. Skin tests and sIgE were measured. Patients were divided into two groups (resting < 2 months and > 2 months).	120 patients with a systemic reaction to insect stings and 100 healthy controls. Ancona, Italy.	There was a significant difference in the distribution of the time interval between the two stings in both groups (p = 0.0001). In 71 of the 120 allergic patients, the provocative sting that provoked SR was essentially the second sting after some previous sting that did not provoke a systemic reaction and occurred less than two months before the systemic reaction. There were only four individuals in the control group who experienced a sting less than two months after the resting. The mean age was lower (34 ± 12 years) in the 71 patients who responded to SR < 2 months after resting than in the other 49 patients (43 ± 14 years). Therefore, age 34 ± 12 years is associated with an increased risk of SR reaction. A comparison of the severity of the allergic reaction after the resting did not show that the severity of the first reaction predicts the severity of the subsequent reaction. Sex and a history of atopy were not associated with the severity of the allergic reaction after the resting. Skin tests and sIgE did not predict the severity of the resting reaction. The type of insect was not associated with the severity of the resting reaction.	The short interval between the two stings is indicated as an important risk factor for the development of SR on insects.
Van der Linden et al. [1994] (27)	Identify a predictor of an anaphylactic reaction after an insect sting.	Prospective observational cohort study.	324 patients with a history of SR-anaphylaxis for wasp stings (272) or bees (52) were invited to the hospital for a provocation test. They considered sIgE measurement, skin tests, severity of previous reaction, sex, age, atopic constitution (history of eczema, hay fever, asthma, allergic reaction to drugs, X-ray contrast agents or food), histamine skin test results, location and number of previous stings, time from previous SR in association with the clinical severity of the reaction after hospital provocation sting test. The severity of SR was assessed according to the Müller's grading system.	324 patients with a history of SR, Utrecht, Netherlands. From 1982 to 1990.	Repeated SR after provocation test with a sting: **Bee:** In 14 (27%) patients, SR was assessed according to the Müller's grading system the same as in the previous reaction. In most patients, SR was less severe or non-existent after the provocation test. **Wasp:** In 31 (11%) patients, SR was assessed according to the Müller test after the provocation test as in the previous reaction. In most patients, SR was less severe or non-existent after the provocation. None of the 324 patients had a more severe reaction after the provocation test than the previous SR. Patients sensitized to bee stings were more likely to have a severe relapse (grade III or IV) (31%) compared to those sensitized to wasp stings (13%). Neither skin tests nor sIgE and sIgG4 were significantly correlated with the rate of allergic reaction in both the wasp and bee sting provocation test, taking into account the overall population. The severity of the previous reaction, sex, atopy, histamine skin tests, placement of the previous sting, time between first reaction and the provocation test, and the number of previous stings – did not correlate with the severity of the reaction after the provocation test. The severity of this reaction was significantly related to age. Patients with severe SR were significantly older on both the bee and wasp venom provocation tests. The time interval between provocation and the onset of the reaction was related to the severity of the reaction. Patients with a more severe reaction developed symptoms and signs more quickly in the provocation test with both wasp and bee stings. Logistic regression results: **Wasps:** the elderly and sIgE levels, only in the population of these elderly patients, pose a higher risk of a recurrent severe reaction. The severity of the first reaction poses an increased risk of a recurrent severe reaction. **Bees:** the elderly and sIgE levels do not pose a greater risk of a recurrent severe reaction. The severity of the first reaction poses a higher risk of reacting again with a severe reaction.	They concluded that the current criteria for assessing hypersensitivity to insect venom (skin tests, sIgE, sIgG4) do not predict the incidence and severity of SR after insect sting provocation.
Bjornsson et al. [1995] (28)	To identify patients at risk for a systemic allergic reaction after an insect sting.	A retrospective observational cohort study.	The study was conducted in 1,815 patients, of whom 52% were men and 48% were women, aged 20 to 44 years. The prevalence of sensitization to bee and wasp venom was assessed by measuring sIgE. Atopy and the presence of other allergic diseases were also considered. From 1,815 patients, 1,399 patients were selected from the general population.	1.815 patients in 3 areas in Sweden. Between 1991 and 1992	Among the subjects, 9.3% had sIgE for bee or wasp venom, 1.5% had SR on bee or wasp stings, and 0.6% had sIgE for both venoms. Sensitization to bee or wasp venom was positively associated with atopy (hay fever, asthma, allergic rhinitis, skin eczema) (OR = 2.0; 95% CI = 1.4-2.8, p <0.0001), with male sex (OR = 1.8; 95% CI = 1.3-2.5, p <0.001) and age (OR = 2.0; 95% CI = 1.4-2.8, p <0.001), and negatively with life in the northernmost part of Sweden (OR = 0.4 95% CI = 0.3-0.7, p <0.001). Atopy has not been identified as a risk factor for a systemic reaction.	They found that the prevalence of allergies in Sweden is not as high as in other countries. People with atopy have a higher risk of becoming sensitized, but do not develop a systemic reaction more often than non-atopic people.

Author, year of publication	The objective of the study	Research epidemiological basis	Methods of data collection	Observed population/ environment/period	Results	Main findings
Blaauw et al. [1996] (29)	To determine whether an insect sting provocation test in a hospital can be used as a criterion for initiating immunotherapy.	A retrospective observational cohort study.	They included patients with a history of severe systemic reaction after bee or wasp stings, with a positive skin test and positive sIgE, in whom a provocation test was performed, which was negative. These patients were followed for 3 years after a negative provocation test. With 479 patients, a new insect sting provocation test was performed. Patients with a negative response to the first provocation were asked about their experiences with stings in the natural environment. Surveys were sent to them and they were called by phone for additional information. The doctor determined the grade according to Müller's grading system.	479 patients (136 sensitized with bee venom and 343 with wasp venom), The Netherlands. Monitoring patients from 1979 to 1994.	Immunotherapy was advised for all patients with a history of severe reaction and a systemic reaction after the first provocation test. **Bees:** Of the 136 patients, a positive response with SR 60 (44%) and a negative response to the first provocation were 76 (56%). The SR grade after the provocation test was significantly different from the previous stings. A statistically significantly higher proportion of patients with a history of severe SR (III or IV) changed to a milder form of SR (II or I) upon provocation. Of the 60 patients who responded to the SR provocation test, 56 received immunotherapy, and the remaining 4 were beekeepers who refused immunotherapy. 2 stopped beekeeping and 2 experienced natural unresponsive stings with SR. All 56 patients with VIT underwent another provocation test six months later. 51 subjects were protected and 5 received further immunotherapy with a higher dose. Of these, 3 became protected, 1 stopped immunotherapy due to side effects, and 1 did not become desensitized. VIT for bees was successful in 96.4% of patients. Of the 76 patients sensitized to bee venom with a negative first provocation test, 41 (53.9%) experienced a subsequent natural sting; 6 (14.6%) had a mild SR (I). A more severe SR (III) than the first in the anamnesis was reported by one patient. Characteristics of patients who did not resting after a negative provocation test and those who experienced a natural resting after a negative provocation were not statistically different in age, severity of SR in history, sIgE, and interval between the sting and the provocation test. However, there were several men in the group with a resting (beekeepers). **Wasps:** Of the 343 patients, 59 (17.2%) had a positive SR response and 284 (83%) had a negative response to the first wasp venom test. The SR grade after provocation was significantly different from the previous stings. A statistically significant higher proportion of patients with a history of severe SR (III or IV) changed to a milder form of SR (II or I) upon provocation. Of the 59 patients with SR, 58 went for VIT. 1 patient resigned without giving reasons. 3 patients withdrew due to severe adverse reactions. All VIT patients underwent a provocation test again after six months. 50 were protected, 5 patients continued higher-dose VIT, and 3 became protected. 1 patient refused further VIT due to side effects and 1 did not become desensitized. Thus, wasp VIT was successful in 91.4% of patients. Of the 284 wasp-sting-sensitized patients with a negative first provocation test, 127 (44.7 %) later experienced a natural sting; 13 (10.2% had SR again, of which 9 (7.1%) SR had a mild (I, II) and 4 (3.1 %) patients had severe SR (III, IV). Of the 127 patients who experienced a natural wasp resting, 5 responded with the same severity as assessed by Müller's grading system and the remaining 122 with a milder reaction than in their previous history. Without the provocation test as a selection criterion for immunotherapy, the proportion of patients treated unnecessarily was calculated to be 45% for those sensitized to bee venom and 74% for those sensitized to wasp venom. However, with a negative provocation test as a selection criterion for immunotherapy discontinuation, 14.6% of bee-sensitized patients and 10.2% of wasp-sensitized patients remain at risk for a systemic reaction at a later sting.	Immunotherapy for bee or wasp venom is justified only after a positive response to the provocation test.
Annila et al. [1996] (30)	Assess the prevalence and type of reaction after bee stings or wasps and to further identify potential risk factors for systemic reactions in beekeepers.	A retrospective observational cohort study.	An online questionnaire was sent to all members (274) of the local beekeeping association. 191 beekeepers (27 females, 164 males) met the inclusion criteria. The presence of atopy was assessed by anamnesis (allergic rhinitis, allergic asthma, atopic dermatitis). The severity of SR was assessed according to the Müller's grading system.	191 beekeepers, Tampere, Finland. September 1993.	A systemic reaction to bee stings was experienced by 50 (26%) beekeepers (13 (I), 15 (II), 15 (III), 7 (IV)) and 73 (38%) beekeepers experienced a large local reaction. The association between patients' history and SR severity was statistically insignificant. 18 of them reacted with SR in the last season and 32, 2 years or more ago. After their last SR in the anamnesis, each subject was stung by a bee between 1 to 360 times. Beekeepers with a history of SR were statistically significantly younger and beekeepers less time than those without SR. 50 SR-responders had more apiaries and fewer stings in 1993 than the other 144, but this difference was not statistically significant. Body constitution and age at onset of beekeeping were not associated with SR severity. 45 (90%) beekeepers with SR used protective gloves. 97 (69%) of 141 non-responders used protective gloves. The difference was statistically significant. Sex and smoking were not statistically significantly associated with SR. 24 (48%) of those who reacted with SR, 39 (28 %) of other beekeepers had a history of atopic disease, which was statistically significantly associated with SR. During work in the apiary, 54% of those who reacted systemically and 23% of the others had nasal and ocular symptoms. Those who reacted systemically were younger and beekeepers for a shorter time. The results of multiple-logistic regression had shown that the risk of a systemic reaction is 4 times higher in the presence of nasal and ocular symptoms, during work in the apiary, and 2 times higher for beekeepers who have been beekeeping for less than 15 years. The SR reaction after the eighth sting was experienced by 2%, or a large local reaction by 13% of beekeepers. Of the 50 systemic responders, 47 did not have a wasp sting. Three beekeepers responded with a systemic reaction to wasp stings. All three also had a history of a systemic reaction following a bee sting.	The occurrence of systemic and large local reactions after bee stings is high in beekeepers. A history of atopy is associated with a systemic reaction. Both; the presence of symptoms in the nose and eyes during work in the apiary and a history of beekeeping for less than 15 years, significantly increases the risk of a systemic reaction.

Author, year of publication	The objective of the study	Research epidemiological basis	Methods of data collection	Observed population/ environment/period	Results	Main findings
Anilla et al. [1997] (31)	Evaluate the usefulness of a structured questionnaire in addition to sIgE values in predicting beekeepers' systemic response to bee stings.	A retrospective observational cohort study.	Participants in previous studies completed a questionnaire on potential risk factors for a systemic response. They measured sIgE and prick skin tests before the beekeeping season. From the history of stings during the season, they assessed reactions to bee stings. A new questionnaire regarding reactions to stings during the season was sent to beekeepers after the end of the beekeeping season. The severity of SR was assessed according to the Müller's grading system.	78 beekeepers (7 females, 71 males), Tampera, Finland. The first questionnaires were sent in September 1993. Second questionnaires were sent in October 1994.	In 1994, 11 (14%) of 78 patients responded to SR after a bee sting. 1 was excluded due to a toxic reaction. 9 (90%) of 10 had pre-season sIgEs higher than 1.0 kU/L, which meant there was a statistically significant connection with SR. Before 1994, 8 (80%) patients had SR, which again meant a statistically significant association with sIgE. The presence of nasal and respiratory symptoms during work in the apiary was statistically significantly higher in the responders than in the non-responders. Positive skin tests, history of atopy, and large local reactions were more common in beekeepers with SR in 1994 than in others, but the differences were not statistically significant. Respondents also had more beehives and fewer stings per year, were younger, and beekeepers less time. An important risk factor is indicated: the presence of pre-seasonal serum sIgE at concentrations greater than 1.0 kU/l increased the risk of a systemic reaction by 12-fold. The risk was 10 times higher in the presence of nasal and/or respiratory symptoms when working in the apiary. If the beekeeper worked in the apiary for less than 8 years, the risk was 9 times higher, and with the anamnesis indicating a previous systemic reaction, the risk of a recurrent systemic reaction was 8 times higher.	The use of a more accurate patient history in combination with laboratory tests, especially measured sIgE, can significantly improve the reliability of the risk assessment for a systemic reaction to bee stings.
Golden et al. [1997] (32)	To determine the natural course of poison sensitization by observing the rate of increase or decrease of sensitization in healthy adults at a follow-up of 5 to 10 years. Clinical significance of these results includes the frequency of systemic reactions to sting during the observation period.	Prospective observational cohort study.	520 volunteers, adult, light industry workers in the suburban area. Two follow-up visits were planned. Three visits were planned for each subject. The first visit was scheduled after five years for all 520 subjects. The second visit was scheduled 2 to 3 years after the first and the third visit 5 to 10 years after the first. 122 subjects did not respond to the invitation to another follow-up. 398 subjects responded at least once.	520 healthy volunteers, Baltimore, United States of America.	Of the 520 subjects identified at the beginning of the study, 122 did not respond to further visits. The follow-up survey was conducted on 398 volunteers (375 early visits and 205 later visits). Of the 375 subjects who came for the first (early) visit, 87 had positive skin tests at baseline. 58 (67%) had positive skin tests after 2.5 years and 29 (33%) had negative skin tests after that time. At the second (later) visit (median of 6.8 years), 11 (20%) of the 54 patients still had positive skin tests and 43 (80%) had negative skin tests. Despite the fact that skin tests became negative, sIgE remained positive at 11 (38%) of 29 at the first visit and 13 (30%) of 43 at the second visit. Skin tests were negative at the first visit (early visit) in 288 of 375 patients and in 151 of 205 patients at the second (later) visit. At the first visit, 23 (8%) of 288 responders that had a negative skin test reacted with a positive skin test. By the second visit, 9 (6%) of 151 responders that had a negative skin test reacted with a positive skin test. Overall, in 398 patients with at least one visit after an average of 4 years, skin tests changed from positive to negative in 44 (45%) patients, and with 98 patients, from negative to positive in 27 (8.7%) of 309 patients. For these 27 patients, the explanations are as follows: 8 had positive sIgE at baseline and 18 were stung by an insect between visits. In 7 patients, there was no reason for a change from negative to positive skin tests. However, with patients who had positive skin tests during follow-up visits, we cannot attribute sensitization to intermediate stings between visits because the frequency of those stings was the same as with those patients whose skin tests became negative. The risk of SR due to a sting was assessed in the correlation of skin tests and elapsed time. In patients with a positive skin test, 65 of them experienced stings and 11 (17%) responded with SR. There was no statistically significant difference in SR frequency between patients who experienced stings earlier than 4 and a half years after the start of the study, or later than 4 and a half years. There were 120 stings without a reaction in the negative skin test group. The risk of SR in patients who had a positive skin test and a negative history of SR existed in 17% of patients. Therefore, 17% is statistically significantly less than 50% with those with a history of anaphylactic reaction, but statistically significantly higher than 2–5% with those who received VIT.	Asymptomatic sensitization to insect venom is common but transient and disappears at a rate of 12% per year. However, the risk of a systemic reaction with a negative history after resting is significant in adults with a positive skin test (17%) and increases if the positive skin tests persist for several years.

Author, year of publication	The objective of the study	Research epidemiological basis	Methods of data collection	Observed population/ environment/period	Results	Main findings
Golden et al. [2001] (33)	To determine the frequency and characteristics of patients with a negative skin test and systemic reaction after an insect sting.	Prospective observational cohort study.	The prevalence of a negative skin test in patients with a history of systemic insect post-sting reactions was assessed prospectively. They also measured sIgE. In patients who signed consent, the results after repeated provocative insect stings were analysed.	307 patients, Baltimore, United States of America.	Of the 307 patients eligible for the study who had a positive history with a provocation sting test, 208 (68%) tested positive for insect venom (concentration above 1µg/mL). In 36 (36%) of 99 patients with a positive history and a negative skin test, the sIgE results were low-level positive (1–3 ng/mL), or the repeated skin tests were positive. SIgE was high-level positive (4–243 ng/mL) in 7 (7%) of those 99 patients. 56 (57%) of these 99 patients had negative sIgE results. A provocation sting test was performed in 51 of these 99 patients. A provocation sting test was performed in 37 of 43 patients with a negative skin test and positive sIgE and in 14 of 56 patients with a negative skin test and negative sIgE. 11 patients with a negative skin test responded with a systemic reaction to the provocation sting test: 2 had negative sIgE and 9 positive sIgE (7 with mild systemic reaction, 4 with moderate systemic reaction). All responders were allergic to wasp stings. In patients with a positive skin test, a sting provocation test was performed in 141 of 196 patients. 30 (21%) responded with a systemic reaction. The frequency of systemic reactions was 21% in patients with a positive skin test and 22% in patients with a negative skin test. 24% in patients with positive sIgE and 14% with negative sIgE. The anamnesis of previous SRs was assessed as a possible factor in the reaction rate. There was no significant difference in the severity of the reaction in the history between patients with positive and negative skin test. In both groups, the history of mild SR was 25%; moderate in 55% and severe in 20% of patients. In patients with a negative skin test, 6 responded with a SR who had a history of mild SR, 4 with a history of moderate SR, and 1 with a history of severe SR. In the two groups, there was no statistically significant difference in elapsed time since the last SR.	The response of skin tests after an insect venom sting may also be negative in those who will react with a systemic reaction. Skin tests may also be negative in those with a history of systemic reaction after an insect sting and may be associated with positive serological tests for specific antibody (sIgE) venom. Skin tests for insect venom should be repeated during serological tests (sIgE).
Celikel et al. [2006] (34)	To define the characteristics of reactions after insect stings and risk factors for the development of a systemic reaction in beekeepers in Turkey.	A retrospective observational cohort study.	A dedicated questionnaire was sent to 1,250 beekeepers in 7 cities in Turkey. 494 completed (39.6%) questionnaires were returned. The questionnaire contained the following questions: demographic data, history of bee stings, type of reaction, emergency room visit in the last 12 months, atopic diseases, smoking, drug and food allergies.	494 beekeepers (489 men, 5 women) from 7 selected Turkish places. Between December 2004 and June 2005.	444 beekeepers (89.9%) with a history of bee sting exposure in the last 12 months. 29 (6.5%) beekeepers experienced a systemic reaction, of which 9 (2%) had an anaphylactic reaction. 28 (5.7%) beekeepers had a history of emergency room visits, but only 5 in the last 12 months. 55% of beekeepers reported more than 100 stings in the last year. When monitoring the systemic response with respect to age and time of beekeeping in the logistic regression model, the results were significantly related to the systemic response: seasonal rhinitis (OR = 4.4; 95% CI = 1.2–11.5), persistent rhinitis (OR = 4.6; 95% CI = 1.2–18.2), food allergy (OR = 7.0; 95% CI = 2.0–25.0), asthma (OR = 8.0; 95% CI = 2.5–25.6) any other atopic disease (OR = 3.3; 95% CI = 1.2–8.7) and two or more concomitant atopic diseases (OR = 10.9, 95% CI = 3.5–33.8).	The incidence of systemic reaction in Turkish beekeepers is low, which may be due to the protective effect of frequent bee stings. The risk of a systemic reaction increases approximately 3-fold in the presence of one atopic disease and 11-fold in the presence of two or more atopic diseases compared to beekeepers without atopic disease.
Guenova et al. [2009] (35)	To examine serum tryptase concentration as a diag-nostic parameter to assess an individual's risk and its impact on the severity of the allergic reaction in the elderly.	Prospective cohort observational research.	Serum tryptase was measured in 274 patients allergic to bee or wasp venom, with a history of systemic reaction, positive skin test, and positive sIgE who visited the dermatology department in Tübingen, Germany. The SR level was assessed according to Müller.	274 patients (153 men, 121 women), Tübingen, Germany. Between 2004 and 2006.	54 patients had a history of grade I SR, 106 of grade II, 66 of grade III and 48 of grade VI according to Müller's grading system. Basal tryptase levels were elevated in 30 (10.9%) patients (> 11.4 µg/l), of whom only 4 (7.5%) were diagnosed with mastocytosis. The severity of SR according to Müller was statistically significantly increased with elevated basal tryptase levels (p = 0.0003). The mean basal tryptase was 4,274 µg/l in grade I responders, while the mean basal tryptase in grade IV responders was 7.18 4 µg/l. Basal tryptase concentrations above 11.44 µg/l were measured in only 7% of grade I and 21.4% of grade IV responders. The risk of severe SR was statistically significantly increased in the elderly. Patients with grade I reaction were on average 38.9 years old, patients with IV. degree 51 years and a half. Sex was not statistically significantly associated with basal tryptase levels. The article did not assess whether recurrent SR was more severe or lesser than the first SR in the anamnesis.	The results confirmed that serum tryptase levels are a risk factor for severe systemic reactions to insect stings. There is evidence that serum tryptase levels increase with age and are thereby an indicator of increased mast cell load or reactivity, which may be partly the source for the reported severe allergic reactions in the elderly. Because these patients are at higher risk for severe anaphylactic reactions, they are potential candidates for immunotherapy.

Author, year of publication	The objective of the study	Research epidemiological basis	Methods of data collection	Observed population/ environment/period	Results	Main findings
Blum et al. [2010] (36)	Assess the impact of total serum IgE and other potential risk factors on severity of allergic systemic reaction after insect sting.	Retrospektivna opazovalna kohortna raziskava	Of the 1,002 patients in the retrospective analysis who had an allergy to insect stings for more than 5 years, 865 reported a systemic allergic reaction, most commonly after a bee or wasp sting, and 137 a large local reaction or toxic reaction. Of the 865, 758 had cIgE, sIgE, and basal tryptase levels available for analysis, along with a history of atopy (skin prick tests for the 14 most common allergens), age, and sex correlated with the severity of the allergic reaction. The severity of SR was assessed according to Müller's grading system.	1,002 patients, Bern, Switzerland. From January 2003 to December 2007.	In the group of 865 patients with a systemic reaction, 256 (33.6%) had a reaction after a wasp sting, 212 (28.1%) after a bee sting, and 290 (38.3%) failed to identify the insect that stung them. Of the 758 patients, 547 had a positive skin test with bee venom and 651 with wasp venom. Of the 758, 519 responded with a severe systemic reaction (grade III or IV) and 239 with a mild systemic reaction (grade I or II). 61% had a double-positive skin test for wasp and bee venom, and 65% had a double-positive sIgE. They were unable to show a statistically significant effect of cIgE on the severity of the reaction (grade IV) with logistic regression models using categorized cIgE values. There was only a slight tendency for lower cIgE levels in patients with grade IV response rate and higher cIgE levels in patients with grade III reaction rate. There was also no statistically significant increase in sIgE in patients who responded with grade IV in the use of categorized sIgE values with logistic regression models. However, a statistically significant association of higher sIgE levels and the response of patients with grade IV response was found in the values for bee venom sIgE when using bivariate analysis with the Wilcoxon test. Statistical analysis showed a positive association of SR severity with older age (> 50 years). The severity of the systemic allergic reaction was statistically significantly associated in patients with basal tryptase levels > 11.4 µg/l ($p = 0.0001$). However, higher basal tryptase concentrations were statistically significantly associated with age. Atopy affected the severity of the reaction. Severe SR (grade III) was reported statistically significantly several times in atopic patients. Atopy was strongly associated with higher cIgE levels. Atopy was not statistically significantly more common in elderly patients.	The association of severe systemic response with lower cIgE is also related to age. The presence of cardiovascular disease and higher basal tryptase levels also have a significant impact on the elderly, and both are risk factors for a more severe reaction.
Richter et al. [2011] (37)	To identify the factors that predict systemic response in British beekeepers and to study visits to a specialist after a systemic reaction triggered by a bee sting.	A retrospective observational cohort study.	A questionnaire was published in the monthly magazine and on the website of the British Beekeepers Association. Information included: demographics, district of residence, history of allergy to bee venom in the family, number of years of beekeeping, number of stings per year, time from start of beekeeping to first reaction, prophylactic use of antihistamines, current atopic diseases, cardiovascular and respiratory diseases, severity of systemic reaction, examination by an allergist, prescribed adrenaline injection and immunotherapy.	852 beekeepers, United Kingdom.	852 beekeepers responded, of which 63% were men, most of them aged 51 to 60 years. 28% of all involved experienced a large local reaction and 21% a systemic reaction. Factors characteristic of beekeepers with a systemic reaction were: female sex, positive family history of insect venom allergy, more than two years of beekeeping before the systemic reaction, and taking antihistamines before the start of beekeeping. 44% of beekeepers with a systemic reaction visited the emergency room, 16% were examined by a specialist and only 8% have an adrenaline self-injector with them at all times.	The analysis identified some new factors related to the systemic response: the levels of emergency room and specialist allergist visits and adrenaline supplies were low, indicating the need to educate beekeepers and doctors and other healthcare professionals.

Author, year of publication	The objective of the study	Research epidemiological basis	Methods of data collection	Observed population/ environment/period	Results	Main findings
Sturm et al. [2014] (38)	To highlight the importance of clinically insignificant sensitization by deliberately provoking a sting test and monitoring serological changes over a period of two years.	Prospective observational cohort study	A sting provocation test was performed in 94 subjects (44 women) with hitherto insignificant (no systemic reaction) sensitization (presence of sIgE for insect venom). In subjects with double sensitization, a provocation test with both points was performed on the same day. After the provocative test, they repeated serological tests. Clinical outcome was correlated with sIgE, skin tests, and basophil activation test. SIgE levels were monitored after three hours, one week, four weeks, and one year. The systemic response was assessed according to Ring and Messemer.	94 subjects, Graz, Austria.	48 (51.1%) subjects had atopy, 27 (28.7%) had a history of large local reaction. Of the 94 provocations, 41 subjects have been stung by a bee, 16 by wasps and 37 by both insects in the past. Only 6 (5.3%) subjects had a systemic reaction (2 per bee sting, 2 per wasp sting, and 1 on both stings), 4 subjects had a grade I reaction, 1 subject grade II reaction and 1 subject grade IV reaction. There is no comparison in the article regarding the degree of severity of the reaction after the provocation test or the previous one in the anamnesis. 41 (43.6%) subjects had a large local reaction after the provocation test, 16 (39%) of them had a large local reaction already in the anamnesis. Compared to the general population, there was a 9.5-fold higher risk of large local reaction, but nothing greater for a systemic reaction. Subjects with a history of LLR did not have a statistically significant higher risk of SR after provocation than subjects without a history of LLR. Of the 37 subjects with low cIgE levels, 1 (2.7%) reacted with SR; of the 29 subjects with moderately high cIgE levels, 1 (3.4%) reacted with SR; of the 28 subjects with high cIgE levels, 3 (10.7%) responded with SR. It follows that there is no statistically significant difference between the different levels of cIgE and SR. sIgE and skin tests after provocation with a systemic reaction for the wasp sting were positive for all subjects. sIgE after a provocation test with a systemic reaction for bee stings were positive in only 1 of 3. The frequency of positive serological tests after negative provocation was not statistically related to SR after provocation. A large local reaction was less common after bee stings, in 26 (33.3%) of 78 subjects, and in wasp stings in 29 (54.7%) of 53 subjects, but the difference was not statistically significant. Three hours after the provocation sting test, sIgE levels were reduced, but in none of the 89 subjects were the results negative. After 1 week, sIgE levels increased (2.2-fold for wasp venom and 2.7-fold for bee venom) and 4 weeks after provocation, by more than 3.5-fold for both venoms. (0.2 to 34.0). In systemic responders, only 3 of 5 cases could be analysed, but the increase in sIgE was similar. To assess the clinical relevance of this increase, 18 subjects were selected for repeated provocation test after one year (16 bee venom, 1 wasp venom, 1 both venoms). Again, 50% had a large local reaction and none had a systemic reaction. The increase in sIgE was similar to that after the first provocation, 4 weeks after the sting. cIgEs were not statistically significantly reduced in either systemic response or large local response agents.	Although sensitization to insect venoms is common, the risk of a systemic reaction for sensitized persons is low. An increase in sIgE after a provocation test is not a true indicator of conversion to symptomatic sensitization. Currently available tests cannot distinguish between a symptomatic sensitization, large local reaction, and systemic reaction.

Legend: LLR - large local reaction, SR - systemic reaction, cIgE - total IgE, sigE - specific IgE, sIgG - specific IgG, cIgG - total IgG, OR - odds ratio, 95% CI- 95% confidence interval, VIT - Venom Immunotherapy.