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Summary

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This thesis gives an overview of some of the main challenges and controversies in the understanding, diagnosis, and treatment of Hymenoptera venom allergy with a special focus on patients with indolent systemic mastocytosis (ISM). We aim to address clinical and biochemical factors related to anaphylaxis and to provide a practical guidance for decision-making in diagnosis and treatment. This chapter presents a summary of the results of our research, the implications thereof on clinical practice and recommendations for future research.

**CHALLENGES IN THE DIAGNOSTIC PROCEDURES OF PATIENTS AT RISK FOR SYSTEMIC REACTIONS TO HYMENOPTERA STINGS**

*Chapter 2* aims to improve the diagnostic sensitivity of conventional yellow jacket venom (YJV) extract (*YJV ImmunoCAP*) by adding the major allergen Ves v 5 in a recombinant form (*rVes v 5*). YJV extract is used as a first-line diagnostic work-up for YJV allergy and consists of a mixture of natural whole venom extracts of several Vespula species. Some of the major allergens such as Ves v 5 have been produced in a recombinant form and are used as a second-line analysis in cases where the causative insect is uncertain or double-positive results for both honeybee and yellow jacket venom are obtained with YJV extract. In the routine use of recombinant allergens we found that IgE reactivity was frequently higher to *rVes v 5* than to YJV extract and that in some cases IgE reactivity was only detectable by *rVes v 5*, suggesting a shortage of Ves v 5 in the conventional YJV *ImmunoCAP*.

To test our hypothesis, we prospectively investigated IgE reactivity to several allergen compounds in a multicenter trial in 308 patients with an established diagnosis of YJV allergy. Amongst others, we compared IgE reactivity to YJV extract, *rVes v 5*, and to a newly developed extract in which *rVes v 5* was added to YJV extract (*rVes v 5*-spiked YJV *ImmunoCAP*). We found substantially higher specific (s)IgE reactivity to *rVes v 5* than to YJV extract (on average 2.4 times higher) and could detect sensitization (≥ 0.35 kUA/L) in 89.9% and 83.4% of the patients, respectively. In addition, 84.4% of the patients that were negative to YJV extract tested positive to *rVes v 5*. Next, we demonstrated that IgE reactivity to *rVes v 5*-spiked YJV extract was substantially higher in Ves v 5-positive sera, whereas no relevant difference in reactivity was observed in Ves v 5-negative sera. The increase of sensitivity from 83.4% to 96.8% was not accompanied by a decrease of specificity (as demonstrated in 51 honeybee venom allergic patients without a history of YJV allergy).

A number of mechanisms could theoretically explain a reduced Ves v 5 immunoreactivity in the extract, compared with the recombinant protein. There might be a true shortage of Ves v 5 protein in the YJV extract because natural venom is a heterogeneous mixture and components are present in widely varying concentrations, or particular allergens such as Ves v 5 might be lost or degraded during processing into extracts. Alternatively, an inefficient coupling of Ves v 5 to the assay’s solid phase or sterical shielding of Ves v 5 epitopes by endogenous ligands of
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Ves v 5 or its attachment to the solid phase may cause a diminished accessibility of Ves v 5 in the extract compared to the recombinant protein.

In conclusion, our results suggest an incomplete capture of Ves v 5-reactive IgE antibodies in the conventional YJV ImmunoCAP® and spiking with rVes v 5 can compensate the absence of IgE immunoreactivity without impairing the specificity. As a result of this study, rVes v 5-spiked YJV ImmunoCAP® (Thermo Fisher Scientific) was introduced for routine diagnostics in June 2012 and the previous conventional YJV ImmunoCAP® was withdrawn from the market after a transitional period.

Chapter 3 illustrates the consequences of YJV sensitization in patients with ISM and proposes a customized reference value for YJV-sIgE in this particular group of patients. Patients with ISM are at high risk for severe and recurrent systemic reactions to yellow jacket stings, and sometimes the first systemic reaction may be fatal. A case of a fatal yellow jacket sting reaction just two months after a large local reaction in a patient with known ISM in our clinic raised the question whether sensitization in ISM patients without a prior systemic reaction should have therapeutic consequences. However, robust data on the natural history of yellow jacket venom allergy and the meaning of sensitization in patients with ISM is lacking. In case reports and small patient groups, demonstration of sensitization in ISM patients with a history of anaphylactic sting reactions is reported to be problematic because sIgE levels are regularly below the reference value to conclude sensitization. This phenomenon may be explained by an increased adsorption of sIgE to the high-affinity IgE receptors on the surface of the expanded mast cell population. We hypothesize that ISM patients who have been stung by a yellow jacket have lower YJV-sIgE levels in general, and that this specific group of patients is in need of a customized reference value.

We retrospectively analyzed 153 ISM patients with a history of a yellow jacket sting and used 313 YJV allergic non-mastocytosis patients as a control. Our results demonstrate that yellow jacket stings often result in systemic reactions in ISM patients and that the first systemic reaction is frequently very severe. Moreover, without venom immunotherapy (VIT), subsequent stings often result in recurrence of systemic reactions and most of these reactions are also severe. As hypothesized, YJV-sIgE levels were considerably lower in ISM patients compared to non-mastocytosis patients and the current threshold (0.35 kU/L) was less frequently reached. Subsequently, we investigated whether the diagnostic accuracy of sIgE could be improved in patients with ISM by customizing the reference value. The current reference value of 0.35 kU/L yields a sensitivity of 77.6% and a specificity of 87.5%. The low sensitivity at the current reference value is alarming since we demonstrated that untreated YJV allergy could have serious consequences in ISM patients. Therefore, we propose a customized reference value in ISM patients at 0.17 kU/L (sensitivity 83.6%, specificity 85.0%).

The question as to whether sensitization in ISM patients without a prior systemic reaction should be treated with VIT remains unanswered. Although asymptomatic sensitization was very rare in our ISM cohort, suggesting that sensitization is more often associated with systemic
reactions in this particular group of patients, the risk on future systemic reactions remains uncertain as prospective analyses are difficult to achieve due to the rarity of the disease, the infrequent occurrence of yellow jacket stings and the unacceptable risk of performing diagnostic sting challenges in ISM patients. The only solution seems to be prospective multicenter collection of sIgE and sting outcome data at the moment of diagnosis of ISM in patients that have not yet experienced a systemic reaction, and after every successive sting in order to gather information on the implications of sensitization. Based on the relatively low rate of asymptomatic sensitization and the high prevalence of severe systemic reactions in ISM patients we recommend discussing the possibility of VIT with all ISM patients exhibiting elevated YJV sIgE, even if they are hitherto asymptomatic, in order to arrive at an optimal individualized management strategy.

In conclusion, the high rate of severe systemic reactions to yellow jacket stings in ISM patients underscores the importance of an adequate sensitivity of sIgE in order to detect patients in need of VIT. The significantly lower YJV-sIgE levels in ISM patients compared to non-mastocytosis patients supports the concept for a lowered customized sIgE reference value in ISM patients.

Chapter 4 compares the ability of baseline serum tryptase (bsT) and baseline urinary histamine metabolites methylhistamine (MH) and methylimidazole acetic acid (MIMA) to discriminate between subjects suspected of having ISM who need a bone marrow biopsy from those who do not. Special attention was given to those that were older than 50 years and had a body mass index (BMI) over 25 kg/m². Because ISM has a significant influence on the diagnosis, treatment, and prognosis of Hymenoptera venom allergy, it is important to accurately recognize and diagnose this condition. Immunohistochemical staining of a bone marrow biopsy is the gold standard for the diagnosis of ISM, but should only be performed if there is a reasonable clinical indication because it is an invasive procedure. BsT is the most commonly used indicator of the need for a bone marrow biopsy, but it does not solely depend on the mast cell load and can be elevated by obesity, older age, and a decreased renal function. We hypothesize that the urinary histamine metabolites may be relatively unaffected by these factors because bsT is a direct product of mast cells while MH and MIMA are indirect products that are produced by the degradation of histamine. The reported absence of a concordance between bsT and the histamine metabolites underscores the hypothesis that there might be a discrepancy in factors that affect the level of these analytes.

We conducted a retrospective data analysis of 194 adults in whom bone marrow investigations had been performed because of a high clinical suspicion of ISM and/or elevated bsT, MH, or MIMA levels. ISM was established in 112 subjects and excluded in 82. Non-ISM subjects had a higher bsT when they were over 50 years and had a BMI over 25 kg/m², while urinary baseline MH and MIMA levels did not differ compared to younger, leaner subjects. As hypothesized, multivariate analysis in non-ISM subjects revealed that age and body weight are independent predictors for bsT, while these factors did not influence MH or MIMA levels. The
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diagnostic accuracy of bsT, MH and MIMA is comparable in the total population suspected of having ISM, while it is limited for bsT in subjects suspected of having ISM over 50 years with a BMI over 25 kg/m². We conclude that MIMA is the best indicator of the need for a bone marrow biopsy in older, overweight subjects suspected of ISM.

In conclusion, age and BMI influence bsT whereas baseline urinary MH and MIMA levels are not influenced by these factors. In subjects suspected to have ISM > 50 years with a BMI > 25 kg/m², MIMA has a greater value compared to tryptase in estimating the need for a bone marrow biopsy. The relevance of this finding is considerable because most clinicians are accustomed to using only bsT as a first indicator of ISM, while overweight and ageing are growing problems currently representing 30% of the population suspected of having ISM.

CHALLENGES IN THE TREATMENT OF PATIENTS AT RISK FOR SYSTEMIC REACTIONS TO HYMENOPTERA STINGS

Chapter 5 explores the effect of eliminating the up-dosing phase of yellow jacket VIT (Pharmalgen®) in terms of elicitation of adverse reactions, clinical efficacy (comparing a sting challenge in this group with a sting challenge in a group of untreated YJV allergic patients), immunological effects (measuring allergen-specific antibody profiles and the allergen-blocking capacity of patient sera by the enzyme-linked immunosorbent facilitated antigen binding assay (ELIFAB)), and treatment costs. Currently, VIT is the only therapeutic option to diminish the risk for severe systemic reactions including anaphylaxis in YJV allergic patients. Yellow jacket VIT is a purified extract of raw YJV, which supposedly still contains all allergens. Therefore, it is implemented in an up-dose fashion with the aim of reducing systemic adverse reactions. However, up-dosing is relatively time consuming and expensive while the intended effect, a reduction in systemic adverse reactions, has never been demonstrated. The rationale of elimination of the up-dosing phase in VIT was to reduce costs, increase patient convenience, and potentially shorten the time needed to reach clinical protection. The original study demonstrating the efficacy of VIT showed that challenges with high doses of purified venom did not induce systemic reactions in untreated patients while a subsequent sting challenge resulted in a systemic responses rate of 58%. This suggests that the allergenicity of the venom extract, injected for VIT, is different from that of the venom, injected directly by a yellow jacket.

We designed a randomized controlled pilot study including patients with an established YJV allergy that were randomized to either yellow jacket VIT initiation by a single injection of 100 μg Pharmalgen® followed by 3 consecutive booster injections every 4 weeks (n = 10), or, alternatively, yellow jacket VIT initiation by a modified rush VIT regimen consisting of 12 updose injections in 7 weeks followed by one booster injection 5 weeks later (n = 8). VIT and sting challenges were performed under constant medical supervision and adverse reactions were ascertained and documented according to predefined clinical and laboratory criteria.
None of the subjects developed a systemic reaction to VIT. The clinical efficacy of both VIT regimens was assessed by a sting challenge. To confirm the validity of the sting challenge, a control group of patients with a previous systemic sting reaction was included that received a sting challenge before starting VIT (n = 20). None of the patients in the treatment regimens reacted systemically to the sting challenge while 20% of the control group did. Although three challenges with negative result are needed in order to definitely exclude a persisting YJV allergy, this outcome suggests that four single 100 μg injections may already desensitize patients to the venom. We conducted additional immunological studies, comparing IgG4 antibody levels, and additionally performing a novel cell-free ELIFAB assay. IgG4 antibody levels rose equally by the two VIT regimens and the ELIFAB assay showed that the allergen blocking IgG capacity was also equally increased, supporting the clinical outcome of the sting challenges. From the non-responsiveness to the sting challenge and the development of allergen blocking IgG capacity, we suspect a comparable efficacy of both VIT regimens.

Direct medical costs and indirect costs were estimated as actual costs from a societal perspective. The 100-μg regimen costs $323.76 per patient compared to $856.52 for the modified rush regimen or $759.94 for conventional up-dosing, which entails a 2.6 or 2.3 fold reduction in costs during the up-dosing phase. Implementation of the 100-μg regimen in 50% of the YJV allergic patients in a population comparable to that of the United States would yearly save $54 million compared to conventional up-dosing. Given the current focus on cost and convenience in health care worldwide, our report may contribute to greater use and acceptance of this important and effective treatment.

Although larger randomized controlled trials are needed to ensure the safety of this approach before it can be implemented in clinical practice, we conclude that initiation of yellow jacket VIT at the maintenance dose might prove to be a time efficient and cost-effective option for selected patients in the future.

**Chapter 6** evaluates which suspected risk factors from clinical practice and previous reports are independent risk factors for VIT failure, as evaluated by the frequency of objective systemic reactions during an in-hospital sting challenge (the gold standard) during the maintenance phase of VIT. Although the efficacy of VIT as a therapeutic measure in general is very high, it is estimated that 16–20% of patients with a bee venom allergy and 5–10% of patients with an YJV allergy are not protected. The efficacy of VIT is thought to depend on a variety of factors, but the relative importance of these factors is unknown as previous studies are small and lack an appropriate adjustment for confounders. Knowledge of risk factors for therapy failure is of importance for modifying treatment strategies (e.g. by increasing the maintenance dose or prolonging the duration of the therapy).

We designed the largest observational retrospective study to date on the efficacy of VIT during the maintenance phase, including 1609 cases (1532 patients of which 77 were allergic to both Hymenoptera species) with an established YJV (n = 1261) and honeybee venom (n = 348) allergy. Patients had experienced a systemic field sting reaction for which they received species
specific VIT and a subsequent in-hospital sting challenge to measure treatment efficacy. Data were collected on various putative risk factors.

104 Cases developed a systemic reaction to the sting challenge performed at the maintenance phase of VIT (49 reactions to a yellow jacket sting (reactivity 3.9%) and 55 reactions to a honeybee sting (reactivity 15.8%)). The median time that had elapsed between commencement of VIT and the sting challenge was 15 months. Systemic adverse reactions were observed in 152 cases (9.4%) during the up-dosing or maintenance phase; 25 of these (16.4%) also experienced systemic allergic reactions to the sting challenge. Multivariate analysis showed an increased risk for VIT failure in patients with a honeybee venom allergy, if a systemic allergic adverse reaction had occurred during VIT, when ACE inhibitor medication was used at the time of the sting challenge, and if bsT was above 20.0 μg/L and/or adult onset of cutaneous mastocytosis was present. Protective were a longer duration of the maintenance therapy, double VIT for simultaneous honeybee venom and YJV, and a double venom dose (200 μg) during maintenance therapy, implemented because of a predefined risk profile of the individual patient. Our study confirmed the importance of factors for treatment failure, which were only reported in small groups before.

There are a few limitations to our study. First, the criteria bsT > 20.0 μg/L and/or adult onset of cutaneous mastocytosis was supposed to likely represent patients suffering from systemic mastocytosis (n = 98). It is not unlikely that part of the patients with systemic mastocytosis were missed due to the criteria for mastocytosis used in the study. In the experience of the expert clinic on mastocytosis in Groningen, a substantial proportion of patients that do not have skin lesions with a bsT ≤ 20 μg/L suffer from ISM, while ISM patients usually experience severe reactions (Chapter 3). Consequently, the impact of systemic mastocytosis on the occurrence of reactions to the sting challenge is presumably higher than we could establish by our study. Second, beta-blocker and ACE inhibitor use were underrated because ACE inhibitors and beta-blockers were often temporarily withdrawn at the time of the sting challenge. Six out of thirty patients who were still on ACE inhibitors experienced a systemic reaction compared to none out of eighteen on beta-blockers. The relatively low rate of ACE inhibitor and beta-blocker use can be seen in the large confidence interval of ACE inhibitors while the impact of beta-blockers might have been missed.

In conclusion, therapeutic success correlates with the duration of therapy, type of venom, and venom dose. Adult-onset cutaneous mastocytosis and/or a bsT > 20 μg/L, systemic allergic reactions during VIT, and ACE inhibitor medication at the time of the sting challenge are significant determinants for VIT failure. The increase in protection with duration of therapy suggests that even during the maintenance phase the protection rate increases with time. Double VIT for YJV and honeybee venom and a high venom dose for groups at risk suggest that these are successful strategies.