CHAPTER 1

GENERAL INTRODUCTION
When inquiring about the beliefs of the general public regarding allergies, several common misconceptions seem to exist. The word “allergy” is frequently incorrectly used as a synonym for all symptoms occurring after an insect sting, ingestion of a culprit food, or related to medication use. Moreover, the general public frequently confuses non-allergic food reactions, such as food intolerances, with food allergies, which can result in an unfounded belief that food allergy is less severe than it truly is. However, what does it really mean?

1.1 ALLERGY DEFINITIONS

An abnormally strong response to a certain substance resulting in symptoms or signs, which is tolerated by the majority of the population, is known as hypersensitivity. (1)

Allergy is defined by the European Academy of Allergology and Clinical Immunology as a hypersensitivity reaction initiated by immunological mechanisms. (1) Allergy frequently arises in the first few months of life, but it can develop at any age. The term allergy is broadly used but generally includes a set of clinical symptoms elicited by immunologic mechanisms, that may be mediated by immunoglobulin and/or through cell-mediated responses. These responses are frequently mediated by Immunoglobulin E (IgE) and these individuals are generally referred to as having an IgE-mediated allergy. (1) Many allergic diseases have a chronic course, however there are ways to treat and manage them and some patients may outgrow their allergies completely, even if severe. (2)

Allergic reactions are elicited by allergens. Most allergens are glycoproteins which cause the allergic response by reacting with the immune system. Allergens may be airborne, such as grass pollen, tree pollen or mite; ingested, as in the case of food allergens; or transferred by stinging insects, for instance yellow jacket, wasp or bee. Another group of allergens are medications, with certain types of antibiotics as common elicitors. (3)

The generation of specific IgE (sIgE) after exposure to an allergen is known as sensitization. (4) However, sensitization without the development of symptoms after allergen exposure, also known as asymptomatic sensitization, is common. Thus, an sIgE mediated allergy requires both the development of symptoms after exposure to an allergen and the presence of sensitization. (5)

Cross-reactivity may occur when an allergen of similar structure to the original sensitizing allergen crosslinks with an antibody and elicits an immunologic response. For example, a birch tree pollen allergen shares structural similarities with a specific hazelnut allergen, which may lead to clinical reactivity to hazelnut in patients sensitized to birch tree pollen. (6)
Atopy is defined as an individual and/or a familiar tendency to become sensitized and produce IgE antibodies in response to exposure of allergens commonly occurring in the environment. Allergic asthma, atopic dermatitis, allergic rhinoconjunctivitis, food allergy and IgE mediated anaphylaxis are examples of clinical disorders considered to fall under the definition of atopic diseases. (1)

1.2 ANAPHYLAXIS

Defining anaphylaxis

Anaphylaxis is defined as a “severe, life-threatening generalized or systemic hypersensitivity reaction”. (1) Anaphylaxis may be mediated by sIgE but alternative mechanisms have also been suggested. (5) An anaphylactic reaction is rapid in onset, varying from minutes to a few hours, and frequently includes multiple organ systems. The skin and mucous membranes are frequently involved, with symptoms and signs such as itching, angioedema, flushing and hives. Gastrointestinal, respiratory and cardiovascular symptoms are also common, however signs of shock is not always present, even in fatal reactions. (7) The overall fatality rate for anaphylaxis is low, under 0.001%. (8) The clinical criteria for diagnosing anaphylaxis, as defined by Sampson et al. (9), are shown in Table 1.

Table 1. The clinical criteria for diagnosing anaphylaxis, as defined by Sampson et al (9). One out of the three criteria needs to be fulfilled to receive the diagnosis of anaphylaxis.

<table>
<thead>
<tr>
<th>Clinical criteria of Anaphylaxis</th>
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<tr>
<td>1. Acute onset of symptoms or signs, with involvement of:</td>
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<td>• <strong>Skin or mucosa</strong> (for example hives; generalized itch, flush or erythema; angioedema) AND one of the following:</td>
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<td>• <strong>Reduced blood pressure (BP) or related symptoms</strong> (for example syncope)</td>
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<td>• <strong>Airway compromise</strong> (for example wheeze, bronchospasm, dyspnea, reduced peak expiratory flow rate (PEFR))</td>
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<td>2. Two or more of the following symptoms after exposure to a confirmed allergen for that patient:</td>
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<td>• <strong>History of severe allergic reaction</strong></td>
</tr>
<tr>
<td>• <strong>Skin or mucosa</strong> (for example hives; generalized itch, flush or erythema; angioedema)</td>
</tr>
<tr>
<td>• <strong>Airway compromise</strong> (for example wheeze, bronchospasm, dyspnea, reduced PEFR)</td>
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<tr>
<td>• <strong>Reduced BP or related symptoms</strong> (for example syncope)</td>
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<tr>
<td>• <strong>In food allergy</strong>: gastrointestinal symptoms (for example vomiting, abdominal pain, diarrhea)</td>
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<td>3. Hypotension after exposure to a confirmed allergen for that patient.</td>
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<td>• <strong>Infants and children</strong>: &gt;30% drop in systolic BP or age specific low systolic BP; &lt;70 mmHg in 1 month-1 year olds, &lt;(70 mmHg +(2xage)) in 1-10 year olds, &lt;90 mmHg in 11-17 year olds.</td>
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<tr>
<td>• <strong>Adults</strong>: &gt;30% drop in systolic BP or &lt;100 mmHg</td>
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12 CHAPTER 1
**Epidemiology**

Anaphylaxis is frequently elicited by foods, medications or stinging insect venoms, but it could also be triggered by an unidentified cause. The distribution of the causes varies with geographic location and the age of patients. Generally, foods and drugs are the most common triggers of anaphylaxis in patients presenting to the emergency department. (8) Foods are the most common elicitor for anaphylaxis in children, while anaphylaxis caused by drugs and stinging insect venom is more common in adults. (8)

Certain external factors, so-called cofactors or augmentation factors, have also been shown to influence allergic reactions. These include the use of certain medications, in particular nonsteroidal anti-inflammatory drugs (NSAIDs); alcohol; exercise; concomitant disease; acute infection; premenstrual status in females and mast cell diseases. (10-12)

**Treatment**

Initial treatment of anaphylaxis consists of an intramuscular injection of adrenaline in the mid-outer thigh, and placing the patient in a supine position with the lower extremities elevated. If there is respiratory distress or vomiting, a position of comfort might be preferred. If indicated, supplemental oxygen, intravenous fluid resuscitation and cardiopulmonary resuscitation should be provided. Antihistamines, glucocorticoids and beta-2 adrenergic agonists should not be used as monotherapy or administered before treatment with adrenaline. Patients not responding to repeated dosages of adrenaline, supplemental oxygen and intravenous fluid resuscitation require intensive care treatment. (13)

**Management and prevention of recurrence**

Patients with previous anaphylactic reactions should be evaluated by a specialist, receive optimal treatment of additional atopic disease and be given information how to manage and prevent recurrences. The patient should be prescribed one or more adrenaline auto-injectors, which must be carried consistently and used if anaphylaxis reoccurs. Avoidance of confirmed triggers and allergen specific immunotherapy should be initiated, if applicable. (13)

**1.3 FOOD ALLERGY**

**General introduction to food allergy**

Food allergy has been defined as an adverse reaction to food, which is reproducible on each contact with the culprit food and mediated by an immunologic mechanism. The clinical symptoms of food allergy involves the skin, gastrointestinal, respiratory and car-
diovascular tracts. (14) Reactions to foods is most commonly triggered by ingestion, but may also rarely occur after inhalation or skin contact. (13)

A thorough clinical history is vital to the diagnosis of food allergy, as it can ascertain the probability of the diagnosis, identify the potential elicitor and suggest the immunological mechanism involved. The clinical evaluation should involve associated atopic disease, such as asthma, atopic dermatitis and allergic rhinoconjunctivitis. Skin prick tests (SPT) and measurement of the level of food-specific IgE (sIgE) are first-line tests to evaluate IgE sensitization. (14) However, asymptomatic sensitization is frequent and these tests in combination with a careful clinical history frequently over-estimate the diagnosis of food allergy. (15-17) Therefore, oral food challenges (OFC) are usually required to make the diagnosis.

The double-blind, placebo controlled food challenge (DBPCFC) is the gold standard test for the diagnosis of food allergy. In this test the patient receives either the placebo or active food on two separate days in random order. The food used during the placebo and active day of the DBPCFC should be indistinguishable from each other in terms of sensory properties. (18)

In order to prevent severe reactions during the test, patients receive the food in increasing dose increments, with a set time-interval between doses. The food challenge is stopped if a clear clinical allergic reaction is observed or if the last dose is ingested without the development of a clinical reaction. Even though life-threatening reactions are rare, staff performing OFCs should be trained and equipped to treat potentially severe allergic reactions and anaphylaxis. (18)

The management of food allergy is divided into short-term and long-term intervention strategies. The short-term interventions are directed at the treatment of acute allergic reactions, such as injection of intra-muscular adrenaline for anaphylaxis. The long-term strategies are employed to minimize the risk of further reactions. This is achieved through patient education, dietary adjustment and prescription of adrenaline auto-injectors, if indicated. (19)

The dietary adjustment should eliminate the culprit food. Patients should be re-evaluated at regular intervals to examine whether they have developed tolerance to the food in question, as unnecessary dietary elimination impairs quality of life and extensive dietary elimination can lead to nutritional deficiencies. (19)
Currently, there is growing interest in immune-modulating treatment options for food allergy, such as sublingual and oral immunotherapy to induce tolerance. However, these are currently not recommended outside of the research setting due to the potential for severe adverse events. (19)

Identifying patients at risk for severe reactions is important for accurate management and targeted prescription of adrenaline auto-injectors. However, accurate identification of these patients is currently not possible, which results in a great deal of uncertainty for patients, caregivers and clinicians.

Scoring of severe food allergic reactions
Various scoring systems for determination of the severity of food allergic reactions have been developed. However, currently there is no consensus among clinicians and researchers on which scoring system to use. The use of a particular scoring system differs per center according to own preferences, research applicability and clinical experience.

Risk factors and co-factors for the severity of food allergic reactions
A correct assessment of the risk of severe food allergic reactions is important for the successful management of patients. Several risk factors have been proposed, however the impact of each factor in the development of severe reactions is unknown. Patients with previous anaphylaxis to food or severe asthma have a higher risk of severe reactions compared to other patients. (20, 21) Moreover, the age of the patients also seems to have an influence on the severity of reactions, with adolescents and young adults generally having the most severe food allergic reactions. (8)

Food allergic reactions do not seem to show a clear dose-response relationship between the ingested dose and the severity of the ensuing reaction. Threshold doses required to initiate an allergic reaction vary between patients, and do not remain stable over time in some food allergic patients. (22) In a unique study, where the food challenge procedure was allowed to continue with additional doses after the initial reaction, many, but not all patients had allergic reactions which progressed to anaphylaxis. (23) It has previously been suggested that dose sensitive patients have more severe allergic reactions, however this has not yet been shown in published research. (24-26) Thus, the precise relationship between dose and the development of severe reactions is currently unclear.

Biomarkers
There are few published studies examining biomarkers for severe food allergic reactions. Currently, there is no biomarker available which accurately can predict severe food allergic reactions in all patients.
Levels of sIgE and SPT wheals to certain foods have been shown to weakly correlate with severe reactions and cut-off values have been developed to make recommendations on when oral food challenge testing is redundant. (27-29) Thus, these cut-offs are more appropriate for predicting clinical reactivity as compared to asymptomatic sensitization. However, they are not clinically useful for prediction of severe reactions in a food allergic population.

In component-resolved diagnostic tests (CRD), sIgE antibodies are measured against individual allergenic food proteins known as major allergens. This test was developed with the prospect to improve the specificity of sIgE testing. (30) The use of this technique has been broadly studied for peanut, and the allergen components Ara h 2 and Ara h 6 have been shown to be predictive markers for severe reactions to peanut. (31) However, geographical differences in sensitizations patterns have been demonstrated for peanut allergy. (32) Moreover, the cut-off levels for these predictors are not applicable to the majority of the peanut allergic population and the impact of these results are controversial. (33) More large-scale studies are needed to confirm allergen components to be predictive of severe reactions for other types of food.

Currently, potential biomarkers for severe food allergic reactions such as basophil activation tests (BATs), baseline serum tryptase (bsT) levels and platelet-activating factor (PAF) and/or PAF acetylhydrolase (PAF-AH) are limited to the research setting.

1.4 YELLOW JACKET (VESPULA SPECIES) VENOM ALLERGY

General introduction to Yellow jacket venom allergy

Vespid and honeybee stings are the most prevalent insect stings in central and northern Europe. Vespula are commonly known as yellow jackets in USA and wasps in Europe. Vespula preferably build their nests in attics, underground or other similar sheltered locations. Only the queens survive the winter, thus larger populations are only seen in the summer and most insect stings occur during that season. (34)

Most venom allergens are glycoproteins and the major allergens in vespid venoms are phospholipase A1 (Ves v 1), hyaluronidase (Ves v 2) and antigen 5 (Ves v 5). (35-37) Some components of the venoms have toxic effects. Generally, toxic reactions are dependent on dose, influenced by the composition of the venom, and only occur after fifty to several hundred stings. (38-40) A single vespula sting releases between 1.7 to 3.1 μg of venom. (41) The venom composition of individual allergens have many similarities, thus cross-reactivity between different species of vespids is common. (42, 43)
Sting reactions can be classified into normal local reactions, large local reactions, systemic toxic reactions and systemic allergic reactions. Normal local sting reactions in non-allergic patients elicit symptoms of pain, erythema and mild swelling around the site of the sting. These symptoms usually fade after 24 hours, but may remain for a few days. (34) Large local reactions have been defined as a swelling larger than 10 centimeters, which persists for more than 24 hours, and rarely includes the presence of blisters. Large local reactions may last for days to weeks and involve eyes, lips or a whole limb. These reactions may also be accompanied by shivering, fever, headaches or general malaise. The pathogenesis of large local reactions is unknown. (34)

A prevalence of between 0.3 and 7.5% of systemic reactions to insect stings have been reported in Europe. (34) Venom sensitization is present in the majority of patients with previous systemic sting reactions. (44) Symptoms of the skin, gastrointestinal, cardiovascular and respiratory tract can occur. One of the most frequently used classifications of the severity of systemic reactions to insect stings was published by Mueller, see Table 2. (45) Symptoms usually develop within minutes after the sting, but can appear hours or rarely even days later. (46) Fatal reactions to insect stings occur, however the incidence is low, between 0.03 to 0.48 fatalities per 1.000.000 individuals a year. (47) However, between 40-85% of patients with fatal reactions to insect stings had no history of previous anaphylactic reactions. (48, 49)

Table 2. Classification of systemic reactions to insect stings according to Mueller. (45)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
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<tr>
<td>I</td>
<td>Generalized urticaria, itching, malaise and anxiety</td>
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<tr>
<td>II</td>
<td>Any of the above plus two of more of the following: angioedema, chest constriction, nausea, vomiting, diarrhea, abdominal pain, dizziness</td>
</tr>
<tr>
<td>III</td>
<td>Any of the above plus two or more of the following: dyspnea, wheezing, stridor, dysarthria, hoarseness, weakness, confusion, feeling of impending disaster</td>
</tr>
<tr>
<td>IV</td>
<td>Any of the above plus two or more of the following: fall in blood pressure, collapse, loss of consciousness, incontinence, cyanosis</td>
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Diagnosis and treatment of Yellow jacket venom allergy

The diagnosis of Yellow jacket venom (YJV) allergy is based on a detailed clinical history of a systemic sting reaction, in addition to clinical sensitization as shown by detection of venom specific IgE in serum and/or a positive skin test. (34) The history is highly important in making a correct diagnosis, since asymptomatic venom sensitization is frequent. (50)

All patients with a history of systemic reactions to YJV should carry adrenaline auto-injectors and be evaluated by an allergy specialist for the possibility of venom immunotherapy.
(VIT). Subcutaneous VIT is an effective treatment which reduces the risk of additional systemic reactions, prevents fatal reactions and improves the quality of life of patients. (34)

**Risk factors for the severity of allergic reactions to Yellow jacket venom**

In patients with a history of systemic sting reactions, a majority will experience a new systemic reaction after a subsequent sting. (51) The risk of developing a systemic sting reaction increases with a shorter time interval between subsequent stings. (52) Conversely, very frequent stings, more than 200 a year, seem to induce tolerance. (53, 54)

Children with a history of previous mild cutaneous reactions have been shown to have a 10% risk of recurrence of systemic reactions after an additional sting. (55) In adults this risk was 14-20% after a history of previous mild systemic reactions and 79% with a history of previous severe systemic reactions. (56, 57) Systemic sting reactions in children tend to be milder than in adults, and elderly patients generally develop more severe sting reactions. (58-60)

Several factors seem to be associated with severe systemic sting reactions. Cardiovascular disease and treatment with beta-blockers or angiotensin-converting enzyme inhibitors have been suggested to be associated with such reactions. However reports describe conflicting results. (61) Patients diagnosed with indolent systemic mastocytosis (ISM) have clonal proliferation of abnormal mast cells and represent a particular risk group for frequent and severe anaphylactic reactions. This is likely to be caused by excessive mast cell mediator release following triggering of mast cells. (62) Moreover, patients with mastocytosis have been shown to have mainly severe or even fatal sting reactions in several case studies. (63, 64)

A strong relationship between reaction severity to insect venoms and elevated baseline serum tryptase (bsT) levels have been shown. The bsT level is thought to reflect the mast cell number and activity, considering that tryptase mainly is produced by mast cells. (65) Additionally, elevated bsT levels in patients without diagnosed mastocytosis have been shown to be associated with severe systemic sting reactions. (66)

**1.5 AIM AND OUTLINE OF THESIS**

This thesis aims at investigating and exploring the multifactorial nature of the severity of systemic anaphylactic and allergic reactions from different perspectives. In this thesis we will explore a new possible biomarker, identify independent risk factors for the severity of systemic allergic and anaphylactic reactions and investigate the genetics of food allergy.
Special attention will be given to examine the relationship between the eliciting dose and the severity of reaction in food allergy.

In the first part of this thesis, we will address the severity of systemic allergic reactions to foods in a pediatric population. In Chapter 2, we will look at a potential challenge in diagnosing food allergy when using the gold standard double-blind, placebo controlled oral food challenge (DBPCFC). This chapter examines whether the DBPCFC, with a 30 minute interval between doses, is safe in patients reporting longer time-intervals between ingestion of the suspected food and the subsequent reaction. Independent factors relevant for the prediction of the severity of food allergic reactions and the relationship between the eliciting dose and severity of reaction will be addressed in Chapter 3. This chapter also examines the influence of using different scoring systems and the impact this can have on predicting the severity outcome. In Chapter 4 we investigate whether patients receiving a high fat matrix in DBPCFCs with peanut have differences in the severity of reaction or eliciting dose, compared to when a low-fat matrix is used. The differences in frequency of clinical reactivity and severity of reaction between peanut and tree nuts is presented in Chapter 5. In Chapter 6 we will investigate the association between a new possible biomarker, apolipoprotein B-100, and the severity of food allergic systemic reactions. Chapter 7 examines a candidate gene for the presence and severity of food allergy, by investigating STAT6 gene variants in children with food allergy diagnosed by DBPCFCs.

In the second part of this thesis we will investigate the severity of systemic allergic reactions to yellow jacket stings in adults. In Chapter 8 we evaluate independent clinical risk factors for the severity of systemic allergic reactions to yellow jacket stings and quantify how much of these reactions may be predicted by the identified factors.

Finally, a summary of the main results of this thesis, general discussion and future perspectives are provided in Chapter 9.
REFERENCES


