From the Department of Women's and Children's Health

Karolinska Institutet, Stockholm, Sweden

WHEAT SENSITIZATION - MORE THAN FOOD ALLERGY

Nora Nilsson

Karolinska Institutet

Stockholm 2015

All previously published papers were reproduced with permission from the publisher.
Cover image by Rebecka Lagercrantz.
Published by Karolinska Institutet.
Printed by E-print, Stockholm, Sweden

ISBN 978-91-7676-161-8

Wheat sensitization - more than food allergy!
THESIS FOR DOCTORAL DEGREE (Ph.D.)

by

Nora Nilsson, MD

Principal Supervisor:
Professor Magnus P Borres
Uppsala University
Department of Women´s and Children´s Health
Division of Pediatrics

Co-supervisors:
Associate Professor Caroline Nilsson
Karolinska Institutet
Department of Clinical Science and Education
Division of Södersjukhuset

Professor Gunilla Hedlin
Karolinska Institutet
Department of Women´s and
Children´s Health

Opponent:
Professor Karin Fälth-Magnusson
Linköping University
Department of Clinical and Experimental
Medicine

Examination Board:
Associate Professor Thomas Casswall
Karolinska Institutet
Department of Department of Clinical Science,
Intervention and Technology

Associate Professor Christina West
Umeå University
Department of Clinical Sciences
Division of Pediatrics

Professor Esbjörn Telemo
University of Gothenburg, Sahlgrenska Academy
Department of Rheumatology and Inflammation
Research

*What takes us back to the past are the **memories***

*What brings us forward is our **dreams***

Maniem mīļajiem,

Un maniem dēliem

ABSTRACT

Background

Wheat is one of the six most common foods responsible for food allergies in children and is introduced into the diet early in life. In children ingestion of wheat can lead to a variety of clinical manifestations, including cutaneous, gastrointestinal and respiratory symptoms. The methods commonly used in clinical practice to diagnose wheat allergy (WA) - medical history, skin prick test and IgE-antibodies (IgE-ab) to wheat are of limited value in diagnosing clinically relevant WA. One reason for this may be that wheat, since it is a grass from the Poaceae family, contains several allergenic proteins, many of which are known to cross-react with grass pollen allergens. An oral challenge test is recommended to confirm WA diagnosis. Since wheat is such a commonly used food, a food restriction can be complicated both for the affected individual as well as for the family.

Aim

The aim of this thesis is to study the utility of different diagnostic methods to identify truly wheat allergic patients. Furthermore we wanted to compare the impact of WA and grass allergy on the quality of life (QoL) of children with diagnosis wheat or grass allergy and their parents'.

Method

Sixty-three children diagnosed with wheat allergy (wheat group) and 72 grass allergic children (grass group) responded to a quality of life questionnaire and provided blood. The wheat group underwent an open wheat challenge where the children were eating increasing amounts of wheat under controlled conditions. IgE- ab against wheat, ω-5 gliadin, low molecular weight glutenin (LMW glutenin), high molecular weight (HMW glutenin) and the α-, β-, γ-, and ω-5 gliadin (gliadin) were analysed. Grass allergic children were analysed for seven grass-specific components (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11 and Phl p12), three wheat-specific allergen components (Tri a 14, Tri a 19 and gliadin), as well as cross-reacting carbohydrate determinants (CCD). Blood samples for CD-sens (basophil activation analysis) were taken for 24 individuals in wheat group. Inhibition test was used to analyse whether there is cross-reactivity between wheat and grass. This is done by to the patient's serum adding an extract of an allergen; antibodies bind to the allergen and then measure the residual activity.

Results

Half of the wheat allergic individuals tolerated wheat at the challenge (non-WA). The IgE-ab level against ω-5-gliadin was significantly higher in the WA compared to the non-WA children. All children in the WA group had IgE-ab against ω-5 gliadin, low respectively high molecular weight glutenin and/or gliadin. We could also see a positive correlation between levels of IgE-ab against these components and the severity of the reaction. A majority of the grass allergic children had low levels of IgE-ab to wheat (median 0.52 kU_A/L) and 87% had IgE-ab to birch. By inhibition we investigated whether cross-reactivity between grass pollen and wheat could be explained by two allergens commonly found in grass, profilin and CCD. These components could explain the cross-reactivity only in a third of cases. Both those with wheat and grass allergies were positive in the CD-sens, with stimulation of wheat and grass. There was a trend to higher CD-sens values to wheat in the WA group compared to the non-WA. Children in the wheat group had generally poorer quality of life than children in the grass group. The parents to the children in the wheat group experienced a significantly poorer quality of life compared with grass group. Children and parents in the grass group had a good agreement in the quality of life questionnaire but not for all domains in the wheat group.

Conclusion

Based on the results of this thesis half of the children with a doctor´s diagnosed wheat allergy seem to avoid wheat unnecessarily. The reason for this may be that they developed tolerance over time or that the individual is falsely diagnosed due to cross-reactivity between wheat and grass pollen. We have shown that analysing IgE- ab against gliadins and glutenins increases the diagnostic accuracy for wheat allergy and distinguishes between those with wheat allergies from those with IgE-ab to wheat due to cross-reactivity with grass. CD-sens with wheat extract has a limited value -but may be useful when individual wheat proteins are used for stimulation. Quality of life was impaired in families with wheat-allergic children compared to families with grass-allergic individuals. This further strengthens the fact that the diagnosis of wheat allergy needs to be improved to avoid elimination diet and concerns about food allergy.

LIST OF SCIENTIFIC PAPERS

I. **Nilsson N,** Sjölander S, Baar A, Berthold M, Pahr S, Vrtala S, Valenta R, Morita E, Hedlin G, Borres MP, Nilsson C.
Wheat allergy in children evaluated with challenge and IgE antibodies to wheat components.
Pediatr Allergy Immunol. 2015 Mar;26(2):119-25

II. **Nilsson N**, Nilsson C, Hedlin G, Johansson SG, Borres MP, Nopp A.
Combining analyses of basophil allergen threshold sensitivity, CD-sens, and IgE antibodies to hydrolyzed wheat, ω-5 gliadin and timothy grass enhances the prediction of wheat challenge outcome.
Int Arch Allergy Immunol. 2013;162(1):50-7

III. **Nilsson N**, Nilsson C, Ekoff H, Pahr S, Borres MP, Valenta R , Hedlin G, Sjölander S.
Wheat sensitization and clinical characterization of patients allergic to timothy grass pollen.
In manuscript

IV. Borres N[*], **Nilsson N**[*], Drake I, Sjölander S, Nilsson C, Hedlin G, Nordlund B.
Wheat allergy impairs health-related-quality-of-life more than grass allergy in childhood.
Submitted

CONTENTS

LIST OF ABBREVIATIONS

APC	Antigen presenting cell
BAT	Basophil activation test
CCD	Cross-Reactive Carbohydrate Determinants
CD	Coeliac disease
CD-sens	Basophil allergen threshold sensitivity
CRD	Component resolved diagnostics
DBPCFC	Double-blind placebo-controlled food challenge
FA	Food allergy
FDEIA	Food-depend-exercise-induced allergic reaction/anaphylaxis
GI	Gastrointestinal Disorders
HMW/LMW	High/Low Molecular Weight
Ig	Immunoglobulin
IgE-ab	IgE-antibody
LTP	Lipid transfer proteins
MA	Molecular allergology
OAS	Oral allergy syndrome
OFC	Open food challenge
PFS	Pollen-FA syndrome
PR	Pathogenesis-related
SPT	Skin prick test
WA	Wheat allergy
WDEIA	Wheat-Dependent Exercise-Induced Anaphylaxis
QoL	Quality of life

1 INTRODUCTION

Wheat is one of the six most common food allergies in children. Together with hen's egg, cow's milk, peanut, fish, soy and nuts it represents over 80 % of all food allergies in children (1, 2).

Wheat is a very common food throughout the whole world and is introduced into our diet early on in life, either through breast-feeding or when the infant begins to eat other food at approximately 5 months of age (3, 4). The extensive use of wheat and related cereals in our daily diet makes cereal-associated allergies a particularly serious problem. Ingestion of wheat allergens can lead to a variety of clinical manifestations, including cutaneous, gastrointestinal and respiratory symptoms. The symptoms can vary from mild to life threatening. Patients with a medical history of food allergy (FA) could be subject to further examinations. The methods commonly used in clinical practice to diagnose WA include an *in vivo* skin prick test (SPT) and/or *in vitro* serological determination of wheat-specific IgE-ab. In contrast to the situation with eggs, milk and peanuts, these tests are of limited value in diagnosing clinically relevant W (5). The correlation between the outcome of an oral wheat challenge (the gold standard) and serum levels of wheat-specific IgE-ab is poor (4, 6, 7), so there are many false positive tests. One reason for this may be that wheat, since it is a grass from the *Poaceae* family, contains several allergenic proteins, many of which are known to cross-reactivity with grass pollen allergens as (8, 9). As a consequence of the false positive tests, too many patients are classified as being wheat allergic and given dietary restrictions. An oral challenge test is recommended to confirm WA. This is a procedure which is time consuming, has to be performed by trained personnel and constitutes a risk for the patients. Recently other diagnostic methods, such as component resolved diagnostics (CRD) and basophil allergen threshold sensitivity (CD-sens) has been developed and have shown promising results (10, 11). Since wheat is such a commonly used food, a food restriction poses severe disruptions both for the affected individual as well as for the family. This has been shown for other types of FA (12-14).

The aim of this thesis is to study different diagnostic methods and their ability to identify truly wheat allergic patients.

Furthermore we studied the impact of the diagnose of wheat and grass allergy by documenting the quality of life (QoL). The terminology used in this thesis adheres to the recommended nomenclature for allergy from WAO 2003 (15, 16).

1.1 FOOD ALLERGY

Prevalence

FA in children is a common disease in a global perspective. The prevalence of food allergies is reported to be up to 6% of young children and 3%–4% of adults (1, 2). However there is a difference in the FA prevalence reported by the patients or their parents compared to confirmed FA by oral food challenge (2, 17, 18). The reported prevalence vary in different study groups, in different geographic regions and due to variations in diet (18). Furthermore the definitions of allergy also vary, which is also an explanation for variation in prevalence. FA in children is a significant public health concern, manifesting through both an increasing prevalence and a changing pattern in the disease expression (19). In recent generations, FA starts earlier in life, with greater severity and persistence into later ages (20). Recently published meta-analysis found a marked heterogeneity in prevalence numbers due to differences in the definition and methodology used. High prevalence numbers was reported for Western and Northern Europe. Boys have a higher risk of developing food allergy and a family history of atopy and atopic dermatitis are also well known risk factors (21).

The term allergy was presented about a hundred years ago as being a change in an individual's immune systems reactivity (22)**. Food allergy** is defined as an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food. **Food allergens** are defined as those specific components of food or ingredients within food (typically proteins, but sometimes also carbohydrates) that are recognized by allergen-specific immune cells and elicit specific immunologic reactions, resulting in characteristic symptoms. Some allergens (most often from fruits and vegetables) cause allergic reactions primarily if eaten raw.

Classification of food intolerance

The terminology used in this thesis adheres to the recommended nomenclature for allergy from WAO 2003 (15, 16).Reactions to food can be classified as toxic or nontoxic reactions. Toxic reactions can occur when foods are contaminated by toxins, or by viruses. Nontoxic reactions are categorized further as immune-mediated or non–immune-mediated (Fig 1) (23).

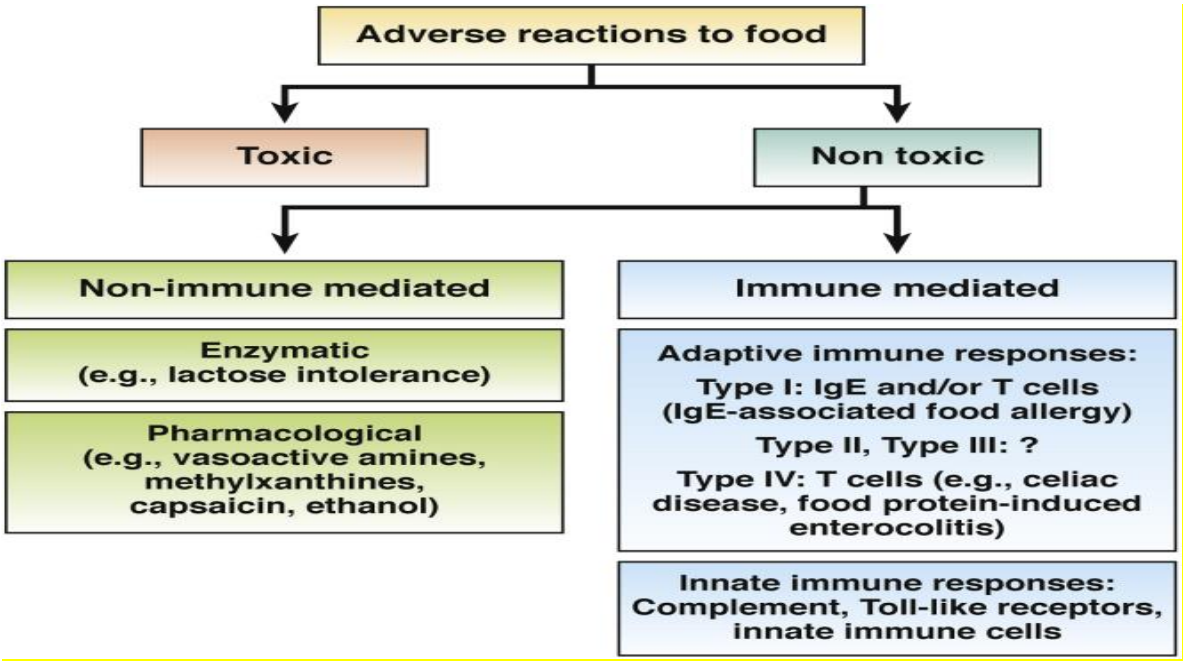

Fig 1.Types of adverse reactions to food. *Adapted from R Valenta (23).*

Non-immune mediated adverse reactions are termed food intolerances and include for instance lactose tolerance. Immune mediated reactions can be divided into IgE-ab mediated and non-IgE-ab mediated conditions such as food protein-induced enteropathy, eosinophilic GI disorders and food-induced allergic contact dermatitis.

Hypersensitivities involving the adaptive immune system can be subdivided into 4 categories (type I–IV). Type I reactions are associated with IgE-ab antibodies against food allergens. There is firm evidence for an involvement of IgG antibodies in type II or type III reactions in immune-mediated adverse reactions to food, whereas type IV reactions involve T cells (celiac disease).

We have in this thesis specifically examined IgE-ab mediated allergy in children and many of them have symptoms from different organ systems (Table 1). About two hundred different foods have been reported to induce IgE-ab mediated reactions. The most commonly associated foods include cow's milk, hen's egg, soy, wheat, tree nuts, peanut and fish (24, 25).

	Immediate symptoms	Delayed symptoms
Cutaneous	Erythema Pruritus Urticaria Morbilliform eruption Angioedema	Erythema Flushing Pruritus Morbilliform eruption Angioedema Eczematous rash
Ocular	Pruritus Conjunctival erythema Tearing Periorbital edema	Pruritus Conjunctival erythema Tearing Periorbital edema
Upper respiratory	Nasal congestion Pruritus Rhinorrhea Sneezing Laryngeal edema Hoarseness Dry staccato cough	
Lower respiratory	Cough Chest tightness Dyspnea Wheezing Intercostal retractions Accessory muscle use	Cough, dyspnea, and wheezing
GI (oral)	Angioedema of the lips, tongue, or palate Oral pruritus Tongue swelling	
GI (lower)	Nausea Colicky abdominal pain Reflux Vomiting Diarrhea	Nausea Abdominal pain Reflux Vomiting Diarrhea Hematochezia Irritability and food refusal with weight loss (young children)
Cardiovascular	Tachycardia (occasionally bradycardia in anaphylaxis) Hypotension Dizziness Fainting Loss of consciousness	
Miscellaneous	Uterine contractions Sense of “impending doom”	

Table 1. Symptoms of food-induced allergic reactions

A diagnose of food allergy leads to recommendations of food avoidance which influences the daily life for patients and their families (26, 27). Naturally this affects their quality of life and furthermore may it increase the cost for the society.

1.2 IMMUNOLOGY

1.2.1 OVERVIEW

The immune system's main function is to protect the body from infection and when an immune cell identifies an affected cell or intruder it does so by reacting to an antigen. These can be relatively harmless, like grass pollen, or harmful like an invasive bacteria (28, 29).

An allergic reaction is the result of that the immune system reacts to a harmless antigen and initiates an immune response to this antigen, which is called an allergen (30).

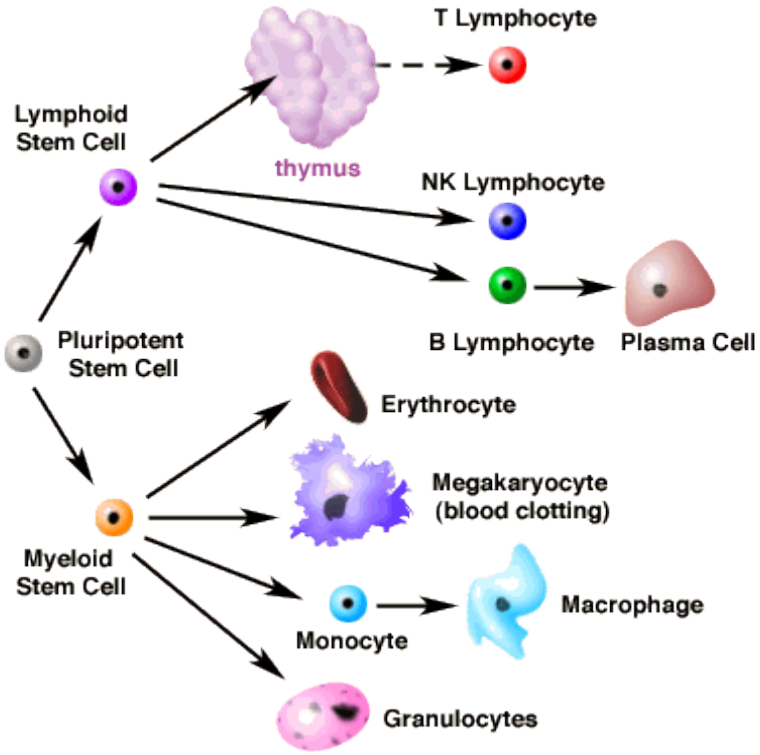

Fig 2. Hematopoesis.

Hematopoietic stem cells produce cells in blood and lymph. Adapted from Biology of the Immune System, JAMA 278.

The main functions of the different immune cells involved in allergic reactions are:

Granulocytes: There are three types of granulocytes; neutrophils, eosinophils and basophils. Neutrophils have the capability to phagocytose bacteria and aggregate in vast numbers around a pathogen. Eosinophils and basophils are important in the defence against parasites. They do so by releasing pro-inflammatory mediators, which also make them important in initiating an allergic process.

Basophils show expression of the activating receptor for IgE-ab (FcεRI), production of Th2 stimulating cytokine and histamine release. Basophils are found in circulation and furthermore they are relatively short lived. Basophils play a role in the delayed hypersensitivity reaction since after the acute phase they

infiltrate the affected organ (lung, upper respiratory tract, skin). *Mast* cells reside in tissues close to surfaces like the epithelium and blood vessels and are one of the major components in the IgE-ab mediated allergic reaction.

Dentritic cells are the most important antigen presenting cells.

T lymphocytes are divided in two types, cytotoxic T-cells and T-helper cells. They play a pivotal role in identifying and killing infected or abnormal cells. They can avoid adverse immune activation, maintain tolerance and prevent immune responses to the body's own cells and antigens.

B lymphocytes, or B-cells, have a specific role to produce antibodies. If activated, the B-cell can multiply and produce huge numbers of antibodies (29). An antibody structurally comprises two elements, two identical light chains and two identical heavy chains see figure 3. The antibody, or immunoglobulin, has two components with different functionality, the Fab and the Fc component. The highly variable part is the Fab component which also is the antigen binding part of the antibody (28). This part is unique for each antibody. The Fc component is the same for all antibodies of a given class and they bind to Fc receptors on the surface of different immune cells.

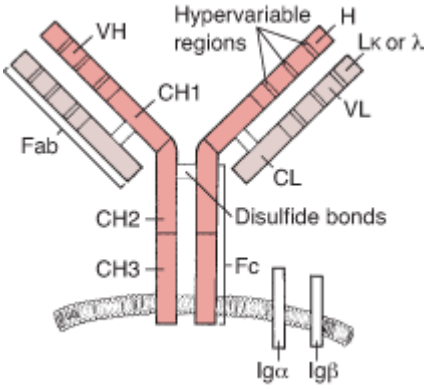

Fig 3. Structure of IgE-ab. *Modified from Delves P.J (28).*

The B-cell receptor consists of an Ig molecule anchored to the cell's surface. CH = heavy chain constant region; CL = light chain constant region; Fab = antigen-binding fragment; Fc = crystallizable fragment; Lκ or λ = 2 types of light chains; VH = heavy chain variable region; VL = light chain variable region.

Five classes of antibodies are known, IgA, IgD, IgG, IgE-ab and IgM. Each class exerts its own specific function in the immune system.

The IgE-ab antibody

The pathological role of IgE-ab in allergy and asthma is now well-established; the normal beneficial function remains unclear. It has been speculated that the primary purpose of IgE-ab could be in the defence against parasitic infections.

Given the ability of IgE-ab to detect minute amounts of foreign proteins in combination with its ability to activate other parts of the immune system, it appears much more probable that it may have a "gatekeeper" function to be the first to sound the alarm when a foreign protein enters the body, activating and recruiting other antibodies and cells that can attack and defeat the intruder (31).

1.2.2 IMMUNOLOGICAL BACKGROUND TO ALLERGIC DISEASE

If the immune system reacts to an antigen which is harmless then an allergic disease may develop.

Allergic sensitization

Allergen contact via the gastrointestinal tract (32), via the respiratory tract (33), and probably via the skin induces IgE-ab production (primary sensitization) (23, 34). Repeated allergen contact activates allergen-specific T cells and induces IgE-ab responses during the secondary immune response. Factors that affect the epithelial barrier and the extent to which allergens are digested or degraded are important for primary sensitization and boosting of secondary immune responses.

The allergic reaction can be divided in an early phase, or immediate, type I reaction and a late phase type I reaction. In the early phase reaction symptoms develop very quickly, within minutes or even seconds. The mechanism behind this is degranulation of mast cells and the release of histamine, proteases and leukotrienes by basophils, causing inflammation and clinical symptoms.

The late phase allergic reaction develops 8-12 hours after the triggering event and is caused by the release of cytotoxic molecules from lymphocytes and eosinophils that have been recruited from the blood into the allergic site (35). This late phase type I reaction should not be mistaken for a delayed hypersensitivity reaction, also known as type IV allergic reaction, which normally develops later, after 2-3 days (23)**.**

Tolerance

Many allergic children develop tolerance with increasing age to foods such as egg, milk and wheat while allergies to peanut and fish tend to persist (36, 37). The higher the IgE-ab level to the food is, the less likely it is that the patient will develop tolerance (25, 38). How fast tolerance develops varies between individuals and allergies and can continue until adolescence. A decline in IgE-ab levels can be an early indication of tolerance development, however it is not always predictive of a true tolerance development since if the individual is not exposed to the allergen then the IgE-ab levels will decline (36, 37, 39).

The reasons why some develop tolerance and others do not are not well understood but several theories have been proposed. The underlying immunological changes include a reduction in allergen-specific IgE-ab production, decreased basophil activation, increased allergen specific IgG_4 and induction of Treg cells (40-42).

1.3 WHEAT ALLERGY

1.3.1 HISTORY

Wheat belongs to the Poaceae family also referred to as true grasses which is a large and ubiquitous group of flowering plants. Wheat is believed to stem from the Middle East and already 9000 years ago primitive wheat was cultivated by humans in eastern Iraq. 7000 years ago it was cultivated in Egypt, China, India and even England.

The importance of wheat throughout history is illustrated in many ways; by ancient descriptions from China how to grow wheat, dating back almost 5000 years and the fact that it is mentioned in the Bible. Socrates said: “No man qualifies as a statesman who is entirely ignorant on the problems of wheat” and a more recent quotation is from President Hoover: “The first word in war is spoken by guns, the last word has always been spoken by bread.”

Wheat is grown on more land area worldwide than any other crop and is currently farmed in all parts of the world. Wheat is easy to grow, transport, store and can be processed into a variety of foods. Wheat contains gluten protein, a feature that is utilized in getting dough to rise because of the small gluten vesicles containing carbon dioxide that are formed during fermentation. Wheat is a component of numerous foods. Breads, cookies, cakes, pancakes, noodles, pasta, ice creams and many breakfast cereals contain wheat and it is also used as a thickener in many foods like soups and sauces.

The structure of the wheat grain

Wheat grains are normally between 5 and 9 mm in length, ovally shaped and weighs between 35 and 50mg. The grains have commonly a red colour and have several compartments as shown in the figure 5.

The protein content varies between 7-17 %.

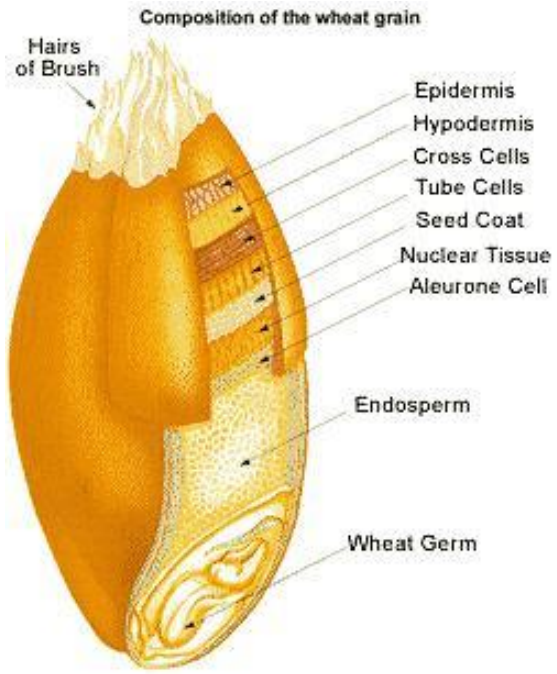

***Figure 5. Composition of the wheat grain*.**

Endosperm is a tissue produced inside the seeds. Inside each cell are found granules of starch and these are surrounded by a clear glassy protein. It is this protein, gluten, when wetted that causes the stickiness and structure of dough. It is this curious combination of properties of the protein that makes wheat flour unique amongst the grains and suitable for bread making.

1.3.2 CLASIFICATION OF WHEAT PROTEINS

Wheat proteins are categorized into four fractions on the basis of their solubility in a series of solvents: water (albumins), dilute salt solutions (globulins), aqueous alcohol (gliadins), and dilute alkali or acid (glutenins). The albumins and globulins are mainly structural proteins and metabolically active enzymes. The water/salt-insoluble gliadins and glutenins, together known as prolamins or gluten, are the major storage proteins of the wheat grain (Fig 6).

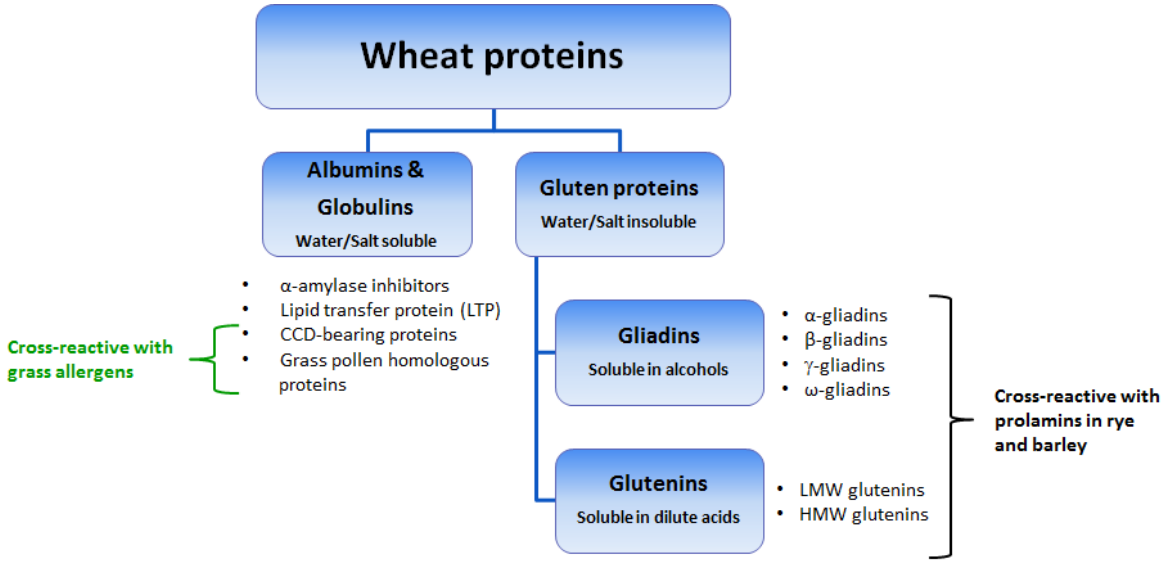

Fig 6. Classification of wheat proteins. *Adapted from Sigrid Sjölander, Uppsala.*

Albumins and globulins

Alfa-amylase inhibitors are the most important wheat proteins contributing to bakers' asthma. Profilins are proteins that are found in all eukaryotic cells and constitute a key component in the cells cytoskeleton. They consist of a large portion of the class 2 allergens (mainly seen in adults and develops as a consequence of an allergic sensitization to inhalant allergens) and frequently show cross-reactivity between pollen and food (43).

Gliadins and glutenins

The prolamins share a great degree of sequence and structural homology with each other and with the corresponding proteins in rye and barley. They can be divided into two fractions according to their solubility in aqueous alcohols: the soluble gliadins and the insoluble glutenins. Gliadins are monomeric proteins and can be classified according to their primary structures into the α/β-, γ- and ω-type. Based on primary structure, glutenin subunits have been divided into the high-molecular-weight (HMW) subunits) and low-molecular-weight (LMW) subunits (44).

Other wheat proteins

Glycoproteins are proteins that contain oligosaccharide chains (glycans) and are often important integral membrane proteins. Water-soluble glycoproteins that are 10 to 70 kDa in size have been identified as a class 1 allergen (the sensitization process occurs in the gastrointestinal tract) (43).

Wheat protein hydrolysates are peptides derived from wheat through proteolysis. These hydrolysates can create allergens of wheat proteins that previously did not exist. Such hydrolyzed wheat proteins is used as an additive in foods and cosmetics.

1.3.3 CLINICAL MANIFESTATION OF GLUTEN RELATED DISORDERS

In 2011 in London, 15 leading experts met and proposed an on new nomenclature and classification of gluten-related disorders (Figure 7) (45) .

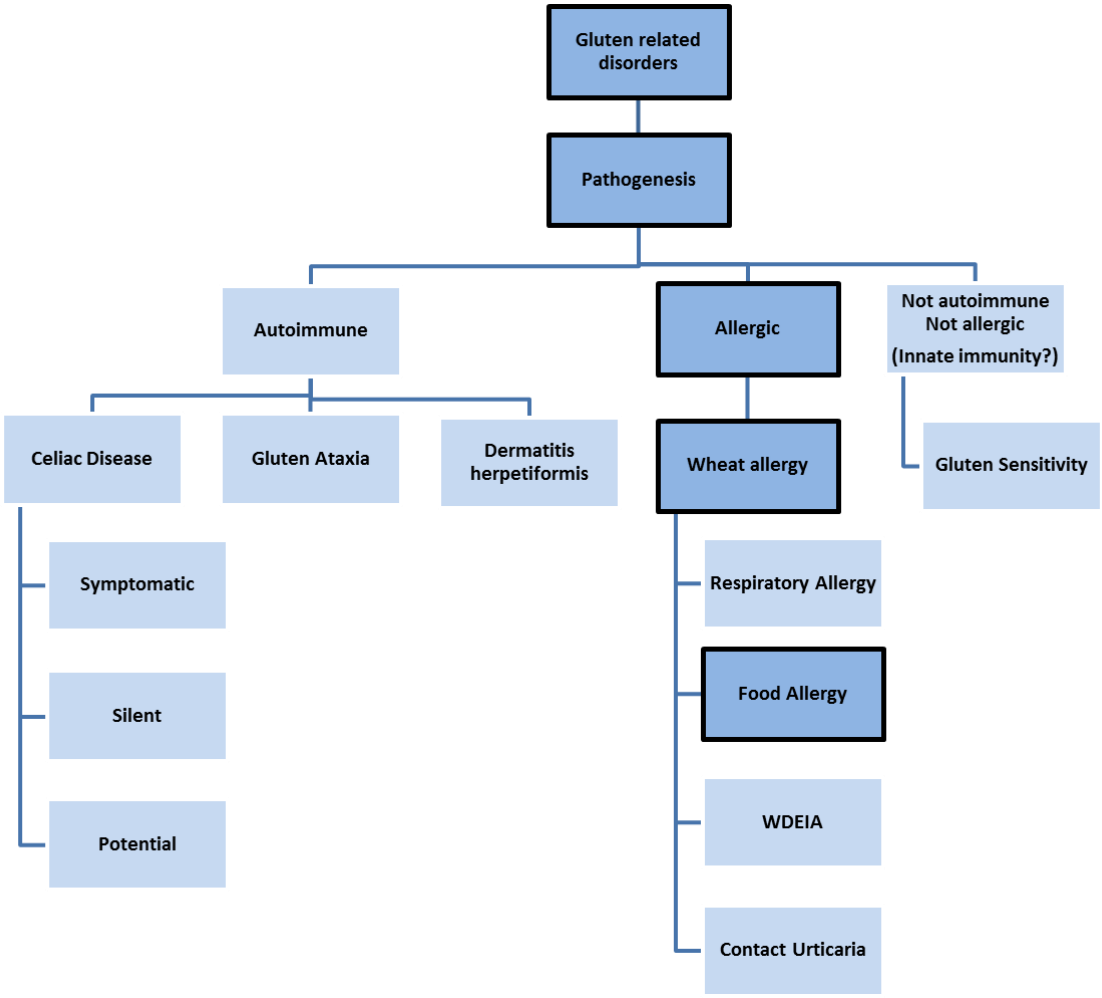

***Fig 7. Nomenclature and classification of gluten-related disorders**. Modified from A Sapone (45).*

1.3.4 WHEAT ALLERGY CLASSIFICATION

Depending on how the individual is exposed to the allergen WA can present itself in different ways;

-Respiratory allergy (Baker´s or occupational asthma and rhinitis)

- IgE-ab mediated food allergy (FA), symptoms from the skin, GI or the respiratory tract

-Wheat-dependent exercise-induced anaphylaxis (WDEIA)

-Contact urticaria

Baker´s asthma

Baker's asthma is an occupational disease affecting bakery workers globally (46) . It is caused by repeated inhalation of the allergen and the annual incidence of disease ranges between 1 and 10 cases

per 1000 bakery workers. It is a severe occupational hazard and wheat flour is the leading causative agent for this allergy.

IgE-ab mediated WA

IgE-ab mediated WA is more common in children than in adults since most children develop tolerance, similar to what is seen with egg or milk allergy (39, 47, 48). However, sensitization as measured by IgE-ab is more prevalent in adults. WA patients present, if wheat is consumed, with characteristic IgE-ab mediated immediate symptoms like urticaria, angioedema, bronchial obstruction, and nausea and abdominal pain, or even systemic anaphylaxis (49, 50). Delayed hypersensitivity symptoms are also seen, which present after about 24 hours and comprise gastrointestinal symptoms and worsening of atopic dermatitis (51). WA in children can elicit all the symptoms associated with FA (Table 1).

Wheat-dependent, exercise-induced anaphylaxis (WDEIA)

WDEIA is an allergic reaction caused by ingesting wheat followed by physical exercise. Since both the amount ingested and the extent of exercise may vary between individuals, WDEIA poses a diagnostic challenge. Furthermore, other factors like NSAIDs (Non-steroidal anti-inflammatory drugs) such as aspirin have been reported to trigger the reaction (52, 53). As the name implies; wheat is the most common agent causing WDEIA. It has also been reported that in some cases more than one trigger is needed to provoke the reaction e.g. exercise and cold stimulation. The reaction lasts several hours and symptoms can vary significantly and can include local or generalized urticaria, dyspnea, hypotension, collapse, and shock (49).

Contact urticaria

Recent studies have found an association between contact urticarial and sensitization to hydrolysed wheat protein (HWP). The fragmentation of gluten is done in order to increase the solubility and the process is known as hydrolysation. This food product is referred to as HWP and has shown to be allergenic. HWP can cause the same reactions as gluten ranging from contact urticaria through cosmetics (54-57) to anaphylaxis when it is used in food or food products (58). Even food-dependent exercise-induced anaphylaxis (FDEIA) has been reported.

1.3.5 WHEAT ALLEGY PREVALENCE

WA and sensitization to wheat is common, but the prevalence varies significantly. Even in children who have been exclusively breast-fed, sensitization may take place. The heterogeneity between studies is high (59). Several factors can explain this, for example there is big discrepancy between self-reported WA and standardized methods. Nwaru reports an overall pooled estimate of WA of 3.6% (95% CI 3.0–4.2) for lifetime self-reported prevalence, 1.5% (95% CI 1.3–1.8) for point self-reported prevalence, 0.7% (95% CI 0.4–1.0) for SPT positivity, 3.9 (95% CI 3.4–4.4) for specific IgE-ab positivity, 0.1% (95% CI 0.01–0.2) for food challenge positivity, and 0.3% (95% CI 0.02–0.6) for food challenge or history of WA (59) . Another factor to consider is at what age the investigations are done as tolerance develops in as much as 65-80 % of patients. Published data also reports geographical differences where WA is more frequent in Northern Europe compared to Southern Europe (60). In a population-based birth cohort in Stockholm, the prevalence of sensitization to wheat in 2336 4-year-old children was reported to be 4% (61, 62).

Wheat allergy - natural history

Published data indicate that 65-80 % of children with an allergy towards cow's milk, wheat, soya and egg, develop tolerance. For peanut and tree nuts the corresponding figure is 10-20 % (42).

Kotaniemi-Syrjanen et al recently reported on 28 children that showed clinical tolerance in in 59%, 69%, 84%, and 96% by ages 4, 6, 10, and 16 years, respectively (39). A US study followed 103 children with a

history of reactions to wheat and sensitization to wheat (47). The percentage of children with tolerance was 29% by 4 years, 56% by 8 years, and 65% by 12 years. In this study the median age by which the patients WA were resolved was 6.5 years. Only a small fraction of the patients had a persistence of their WA into adolescence. Czaja-Bulsa et al reported that the mean age of tolerance was 69.5 months in their WA cohort (48). The rates of resolution were 20% by the age of 4 years, 52% by the age of 8 years, and 66% by 12 years, and 76% by 18 years.

1.4 DIAGNOSTIC METHODS IN WHEAT ALLERGY

Detailed clinical history is essential for the diagnosis of FA and EAACI has recently issued guidelines regarding FA focusing on diagnosis and management (63) . A clinical history of FA includes inducing allergens, timing and chronicity, symptoms, severity and signs, known risk (co)factors, family history, coexisting medical problems including other allergic diseases. It is recommended to use standardized questionnaires to identify these factors.

Different tests and procedures should be used to identify allergens: elimination diet, skin prick test (SPT), IgE-ab measurements in serum, oral food challenge (OFC) and component resolved diagnostics (CRD) as well as CD-sens.

1.4.1 ELIMINATIONS DIET

An initial strategy when treating FA patients is to avoid the suspected food. The decision which food to avoid is usually based on the patient's rapport and the result of allergy tests (SPTs and/or IgE-ab). Only one food at a time should be avoided for a period of 2-4 weeks. If no relief of symptoms is noted it is unlikely that the patient is allergic to that specific food. If, on the other hand a significant reduction in symptoms occur the avoidance should be maintained until a provocation test is done.

1.4.2 SKIN PRICK TEST (SPT)

Wheat is introduced in the skin to confirm whether the patient is sensitized or not. The test is deemed as positive if the diameter of the wheel exceeds 3 mm (64-67). The size of the wheal correlates relatively well with the likelihood of WA (66, 68). The benefit of the test is that it is easy to perform, relatively painless and gives a quick readout. Several factors influence the quality of the test like the skill of the person applying the test, the type of allergen used, the age of the patient, antihistamine intake, pregnancy and skin reactivity (65). Different batches of commercially available allergen extracts can also vary in the protein content which also will influence the result (69-71). Adverse reactions in children have been described (72).

1.4.3 IgE-AB MEASUREMENTS IN SERUM

IgE-ab assays detect and measure circulating IgE-ab that bind to specific allergens in a patient´s serum. Several commercial tests are available and the ImmunoCAP test is mostly used in Sweden. The read out unit is kU_A /L (kilo international units of allergen specific antibodies per litre). One international unit corresponds to 2.42 ng of IgE-ab and the lower limit of detection is 0.1 kU_A /L (ImmunoCAP).There is no interference by anti-histamines or dermatitis, and furthermore several anti IgE-ab can be tested in one sample and the test is standardized (68, 73). The drawback is that venipuncture is needed and the physician receives the result the following days. SPT and serum IgE tests have similar properties and are sensitive. Similar to SPT the result should be interpreted with caution especially when it comes to WA. Sampson et al (5) reported specific threshold levels for a number of major food allergens in a pediatric population, including wheat. The 95 % decision point for wheat was > 100 kU_A /L. The relative incapacity of the IgE-ab tests might be explained by the fact that extract based tests lack important allergens primarily from the gliadin fraction since those are insoluble.

1.4.4 COMPONENT RESOLVED DIAGNOSTICS

CRD, also known as molecular-based allergy diagnosis, involves using purified, native or recombinant allergen to detect IgE-ab sensitization to different proteins in an allergen source (74, 75). The reason for utilizing this technique is that an allergen, like wheat, contains many different proteins which can trigger an allergic reaction, and hence using single proteins to quantify the IgE-ab enables a more specific answer to what is causing the symptoms.

The individual allergen components are named according to the system developed by Linné. The latin name of wheat is Triticum aestivum and the principle by which the components are named is using the first three letters in the genus name followed by the first letter of the species name and finally a number is added for the different allergens identified (i.e. Tri a 1).

The International Union of Immunological Societies Allergen Nomenclature Subcommittee Database has 21 proteins listed as allergens for wheat out of which 9 are linked to FA (www.allergen.org).

Wheat contains many different proteins some of which belong to protein families found also in other plants, such as the closely related grasses (44). Hence an IgE-ab antibody can bind to a protein of another source than the original sensitizing protein (primary allergen) and elicit an immune response. This is known as cross reactivity.

The wheat-specific allergen components can be divided in two groups, the water/salt soluble fraction containing albumins/globulins and the water/salt insoluble fraction consisting of glutenins and gliadins (Fig 6).

Albumins and Globulins

Profilins are known to be the one of the major allergen present in tree, grass, and weed pollen. About 20% of individuals allergic to pollen are sensitized to profilin and it has reported that the IgE-ab antibodies to pollen profilins are highly cross-reactive to profilins in vegetable. Patients often become sensitized to the inhaled pollen at first. If the person then ingests raw fruits or vegetables containing profilins, they can experience oral and pharyngeal symptoms, i.e., the pollen-FA or oral allergy syndrome. The profilins are highly heat labile, susceptible to enzymatic degradation.

Alpha amylase inhibitors are well-known allergens in Baker's asthma (76) and they seem to play a role in ingested WA as well (77, 78). Recently Mäkelä (79) showed that sensitization to alpha amylase inhibitors seem to distinguishing children with immediate reactions from non-reactors.

Lipid transfer protein in wheat and other cereals have also been identified as food allergens in several studies (78). Pastorello et al identified LTP as a major allergen in wheat FA in Italian patients (76, 78).

Glutenins and Gliadins

Gluten proteins are major anti IgE-ab in triggering celiac disease as well as IgE mediated WA (80).

Wheat ω-5 gliadin has been identified as the major allergen in wheat-dependent, exercise-induced anaphylaxis (WDEIA) (4, 49, 81). Several studies have shown ω-5 gliadin to be a significant allergen in young children with immediate reactions to ingested wheat (4, 78, 81, 82).

Hoffmann found high prevalence of specific IgE-ab to omega 5-gliadin, alfa, beta, gamma gliadin and high low molecular weight (HMW) glutenin in patients with WDEIA (83). Pastorello et al reported both alpha and gamma gliadins to be of importance in WA (78). Mäkelä et al reported alpha gliadin to be the best

allergen in the gluten fraction in terms of both sensitivity and specificity with regards to immediate reactions to wheat (79).

Both high and low molecular weight glutenin seem to play a role in WA. Baar et al showed that 80% of WA patients had sensitization to LMW glutenin (84). LMW-glutenin has been reported as major allergen in patients with GI symptoms after ingestion of wheat (77). Takahashi et al reported HMW glutenin to be of significance in Japanese patients diagnosed with WDEIA (85). Mäkelä et al reported on the importance of HMW glutenin in children with immediate reactions to ingested wheat (79).

Cross-Reactive Carbohydrate Determinants (CCD)

Cross-reactive carbohydrate determinants (CCD) are protein-linked carbohydrate structures known to be related to allergen cross-reactivity. Their biological role and activity has been questioned and normally diagnostic results caused by CCDs are therefore regarded as false positives.

1.4.5 CD-SENS

Basophil activation test (BAT) is an *in vitro* test measuring the activation of basophils upon stimulation with allergens (86-88). The activation can be measured in two different ways, either by investigating secretion of mediators from basophils (mediator release assay) or by detecting expression of cellular markers after stimulation (flow cytometric assays). The most commonly used mediator release assays, the histamine release test, measures the release of histamine based on the fact that the granule of basophiles contain histamine. The activation of the basophils is calculated as the histamine released as a percentage of the total histamine content (89).

In flow cytometric assays the evaluation of basophil activation is measured through the detection of unique membrane markers expressed only after activation, such as CD63, or through the increase of the expression of CD203c (89, 90).

In a non-activated basophil CD63 is only found in the intracellular granules and only low levels of CD203c is expressed on the cell surface (Fig 8) (90, 91). When activated granules move to the cell membrane and fuse, CD 63 will be presented on the cell surface. CD203c is rapidly up-regulated when the basophil is activated.

The majority of patients are responsive to activation of their basophils. Published reports indicate that between 80-90 % are responsive (91), implying that as many as 10-20% are non-responsive.

In this thesis we have used a method known as the basophil allergen sensitivity test (CD-sens) (92). It measures the lowest allergen dose at which a 50 % activation of CD63 of the basophils takes place. The lower the level of allergen is the higher the level of allergen sensitivity.

Published studies indicate a relative good correlation between the outcomes of SPT, IgE-ab levels and CD-sens in allergic rhinitis. Pignatti et al report that for FA patients the agreement (positive/positive and negative/negative) between single BAT and SPT was 78.5% and between single BAT and IgE-ab 78.3% (93).

Glaumann et al shows a good correlation between CD-sens and OFC for peanut allergy (10) .

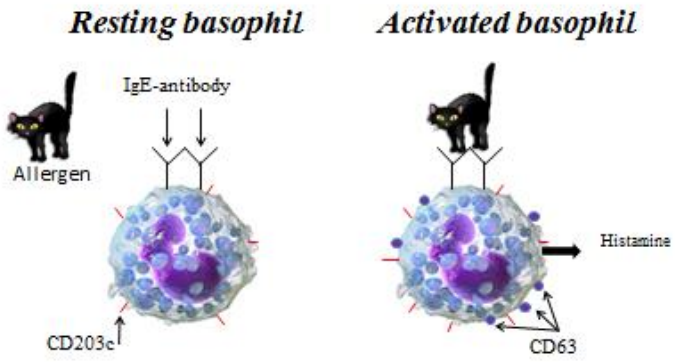

Fig 8. Activation of basophils. *With permission from Anna Nopp (91).*

1.4.6 ORAL FOOD CHALLENGE

To confirm a diagnosis of an IgE-mediated FA it is often necessary to perform a food challenge test (94). It is used to either confirm allergy or to demonstrate tolerance. Patients are given increasing amounts of the allergen in question every 20-30 minutes. If a reaction occurs the challenge is discontinued (95). A scoring system is used to judge the severity of symptoms (95). The result guides the recommendations given to the patient regarding safe dietary expansion or appropriate allergen avoidance.

There are several different types of food challenge tests that may be clinically indicated: open, single-blind, double-blind and/or placebo-controlled. Open food challenge (OFC) is unmasked, non-blinded feeding that is usually done if symptoms such as asthma or urticaria are anticipated (96, 97). OFC is a cost-efficient procedure that saves time and resources (94, 97). In daily practice a non-blinded open food challenge (OFC) is used since it is less time consuming and the food is ingested in its natural form.

Placebo-controlled challenges may be administered in both single-blind and double-blind fashion (97). The gold standard is the double-blind placebo-controlled food challenge (DBPCFC) (95) which should be used when symptoms are subjective or if the symptoms are atypical or if the patient/parents or health care personnel are anxious (95). Furthermore it is the preferred method in research and for selected cases in clinical practice (94, 97).

The challenge food is mixed with the vehicle and administered with increasing dose every 15-30 minutes. This time interval is chosen because most acute reactions occur within this time frame; in patients with a previous history of severe reactions, a lower starting dose is recommended. Initial dose is guided mainly by clinical assessment of risk of reaction and type of FA.

OFC is usually started with 0.1% to 1% of the planned total dose of challenge food. The European Academy of Allergy and Clinical Immunology proposed an initial doses of 100 mg wheat for OFC (71).The most sensitive patients may react at the first 10 mg to 100 mg dose of the challenge food according to published studies (94, 95, 98).

All oral food challenges should be performed under strict medical supervision with emergency support directly available and if there is risk of a severe reaction then intensive care support must be immediately available. The OFC is negative if the patient tolerates the entire challenge, including the observation period 1-2 hours. In case of a positive OFC, the treated patient should remain under observation after symptoms have resolved for up to 4 hours.

1.5 GRASS ALLERGY

Grasses covers a substantial part of the earth's landmass and their pollen is one of the major causes of IgE-ab mediated allergic disease (99). Pollen that gives rise to allergic symptoms are present in the air in northern Europe at least 6 months every year. It causes severe implications for children affected, and interferes in many aspects of their daily lives both physical, practical and emotional (100).

Amongst the grasses, timothy (Phleum Pratense) is one of the most common species to elicit an IgE sensitization. The proteins of timothy has been examined and characterized and as a result a number of allergen components have been identified. Some of which are" species specific allergens" like Phl p 1 and Phl p 5 whilst the profilin Phl p 12 and the calcium-binding protein Phl p 7 are the principal cross-reactive components (101, 102).

Allergy to grass pollen is reported to increase amongst children worldwide and it has also been demonstrated that Phl p1 is the most relevant sensitizing allergen detectable at all ages and at all levels of timothy grass pollen-specific IgE-ab. In contrast, Phl p 5 becomes increasingly more important with the increase of patients' age and with grass pollen IgE-ab levels.

Since both wheat and timothy are members of the Poaceae family they share similar proteins. This is the reason why co-occurrence of IgE-ab to timothy pollen and wheat are common (9). It is uncertain how frequent sensitization to wheat is among grass pollen allergic patients. Furthermore it needs to be elucidated which allergens cause the serological cross reactivity.

Constantin et al reported that 65% of the patients with grass pollen allergy had false-positive IgE-ab test results to wheat (103). In a study of young asthmatics sensitized to timothy. Patelis found that 17% were also sensitized to wheat but only 7% reported wheat hypersensitivity (104).

1.6 HEALTH RELATED QUALITY OF LIFE (HRQOL)

DunnGalvin has defined HRQL as the patient´s perception of the effects of the consequences of a specific disease or the use of a certain treatment on different aspects of his/her life, particularly the consequences for the patient´s physical, emotional and social wellbeing (105).

In order to evaluate an individual's QoL, validated instruments are used. These are often questionnaires rating health and different domains, but may also be a graded scale or a visual analogue scale.

Most instruments define HRQoL with two main domains: physical function and psychosocial function. Since Qol comprises many different aspects, two different types of HRQoL are used: the general instruments (generic) or disease-specific instruments.

Generic instruments asses the general health and can be used to compare different conditions or against general population. Disease specific instruments can be used for specific conditions and questions are specific for that disease.

It has been reported that children suffering from food allergy show lower HRQL scores compared to non-allergic children (106). Even families to patients are affected which was demonstrated by Mikkelsen et al

(107). They studied HRQL among children with cow's milk allergy and found that affected families experienced higher stress on their daily lives and nutrition concerns compared to control families (107).

Protudjer et al (108) recently reported the benefit of using disease specific questionnaires since such instruments can elucidate the impact on QoL which a generic instrument cannot. Furthermore a disease specific questionnaire can distinguish between food allergic impact and impact from comorbidity (109) .

2 OBJECTIVES

2.1 GENERAL OBJECTIVES

The general objective of this thesis was to characterize wheat allergy in children clinically and serologically, and investigate cross reactivity between wheat and grass allergy.

2.2 SPECIFIC OBJECTIVES

The specific objectives were:

- To determine what proportion of children with a medical history of wheat allergy and IgE-ab antibodies to wheat exhibit clinical symptoms in response to an oral challenge with wheat.

- To determine the utility of IgE-ab antibodies to wheat and wheat- and grass-allergen components to predict allergic symptoms in children with a doctor´s diagnose of wheat or grass allergy.

- To determine whether basophils stimulated with wheat, omega-5-gliadin or timothy grass pollen can predict allergic symptoms in individuals sensitized to wheat.

- To analyse possible serological cross-reactivities in children with a doctor´s diagnose of wheat and grass allergy and to compare the quality of life of these two groups of children.

3 MATERIAL AND METHODS

3.1 STUDY GROUPS AND STUDY DESIGN

This thesis includes subjects from two different groups. The first two papers (I and II) are based on subjects with doctor´s diagnosed wheat allergy from Astrid Lindgren's Children's and Sachs' Children´s and Youth Hospital, Stockholm, Sweden (wheat group) (Fig 9). Paper III includes subjects with doctor´s diagnosed grass pollen allergy (grass group). Paper IV includes patients from both groups.

Figure 9 is describe included and excluded individuals from the two study groups and their representation in the different papers.

Astrid Lindgren's Children's and Sachs' Children´s and Youth Hospital are two children´s hospital in the Stockholm Area. The Allergy departments have food allergy units diagnosing and treating children with moderate to severe food allergy.

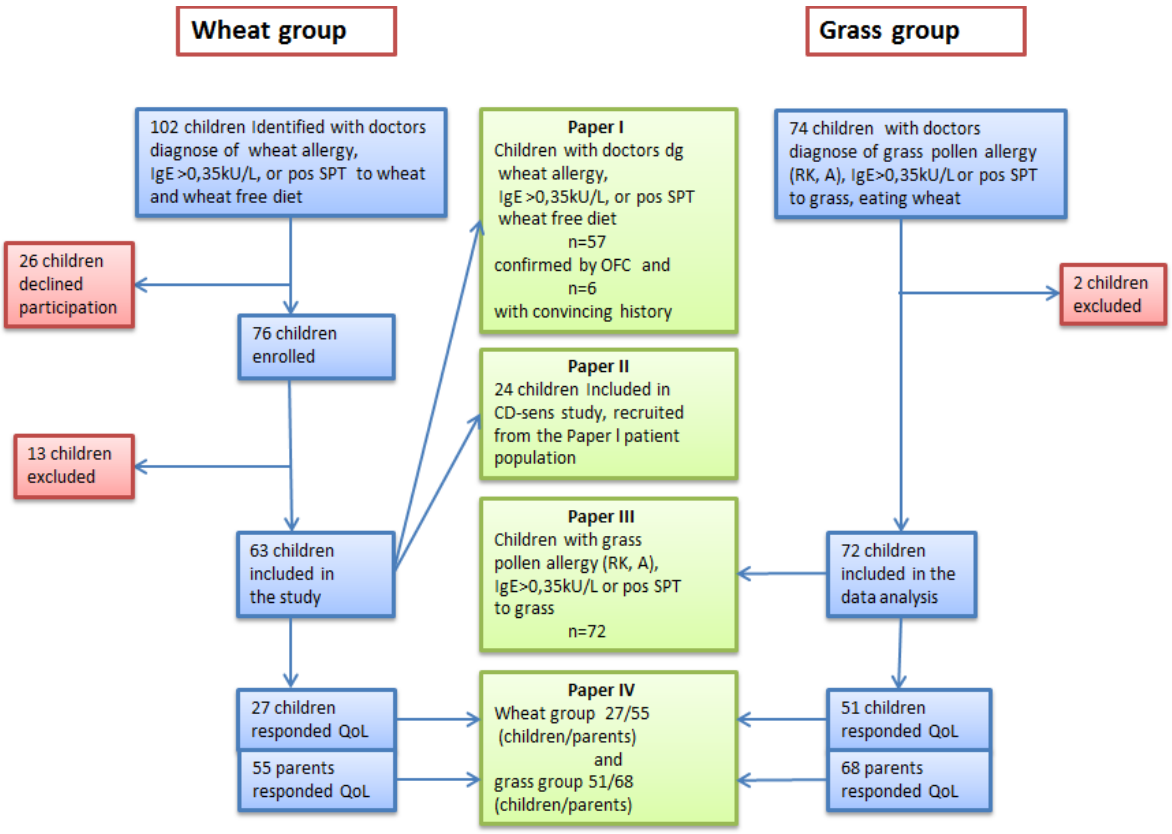

Fig 9. The studied wheat and grass group in relation to the four different articles in the thesis.

3.1.1 Wheat group

To identify patients with wheat allergy the code Z91.0E according the International Classification of Disease, Tenth Revision (ICD-10) were searched for at all children´s hospital hospitals in Stockholm; Sachs' Children´s and Youth Hospital and Astrid Lindgren´s Children's Hospitals Solna (ALB) and Huddinge and in all paediatric open-care units (n=25) in the Stockholm area between 2008-2012. All records with the diagnosis Z 91.0E had to be reviewed in detail as this diagnosis accounts for all food allergies but milk, egg, nuts, peanuts, and fish that have their own diagnosis codes.

Paediatric patients who have received the diagnostic code Z 91.0 E and who fulfil the criteria for inclusion without demonstrating any of the criteria for exclusion were contacted and asked to participate.

Eligible patients were contacted by telephone and were given a verbal description of the study. Those interested in participating received written information and were later contacted again by telephone to ask if they still wished to participate. Children, who were willing to participate in the study and who mailed a written parental consent were scheduled for a visit. All procedures, blood samples and oral food challenges (OFC), were carried out during three visits (Table 2) at the Lung-Allergy Department of Astrid Lindgren´s Children's Hospital, Karolinska University Hospital in Solna.

Inclusion criteria:
- Doctors' diagnosis of wheat allergy.
-IgE-ab to wheat (> 0.35kU$_A$/L) and/or a positive SPT to wheat (>3mm).
-Wheat-free diet.

Exclusion criteria were:
- Celiac disease or other autoimmune diseases.

3.1.2 Grass group

Children with a doctor's diagnosed grass pollen allergy and who had received the diagnostic code J30.1 and/or H10.1 and/or J45.1 and/or J45.8 (Allergic conjunctivitis and/or allergic pollen rhinitis and/or allergic asthma) in ICD-10 were identified in the same paediatric hospital and their corresponding outpatient clinics in Stockholm, where the wheat allergic patients were found.

All children, who fulfilled the inclusion criteria and were willing to participate in the study, were invited to a visit at ALB or Sachs' Children's and Youth Hospital, Stockholm, Sweden. Blood samples were drawn and a QoL questionnaire was filled in at the visit.

Inclusion criteria:
-A doctor's diagnosed grass pollen allergy, IgE-ab to timothy (≥ 0.35kU$_A$/L) and/or positive wheat skin prick test (≥ 3 mm).

- Clinical symptoms during the grass pollen season.

Exclusion criteria:
-Wheat free diet.

-Allergy to wheat.

	Wheat group N=63	Grass group N=72
Visit 1		
Signing the consent form	X	X
Presentation of general information	X	X
Recording the patient's history of allergy	X	X
Clinical examination	X	X
Taking blood samples	X	X
Filling out QoL forms CHQ-CF87and CHQ-PF28		X
Visit 2		
Taking blood samples for determination of CD-sens (N=24)	X	
Performance of an OFC wheat (N=57)	X	
Filling out QoL forms CHQ-CF87,CHQ-PF28, FAQLQ-CF and FAQLQ-PF	X	
Follow-up		
Filling out QoL forms FAQLQ-CF and FAQLQ-PF	X	

Table 2. Study design wheat and grass group.

3.2 STUDY METHODS

3.2.1 Oral food challenges

The oral food challenges performed were done by experienced personnel with knowledge about acute treatment care at allergic reactions and before the challenge, medical treatment was prepared. All challenges were performed at department of Astrid Lindgren´s Children's Hospital with access to an emergency care unit if needed.

The children had to be healthy at time of the challenge and no challenge was performed if the child had an on-going infection or an on-going allergic reaction to other food or inhalant allergens. The challenge was also postpone if the child had used antihistamine less than 4 days or oral steroids < 2 weeks prior to the challenge. A medical history with focus on wheat allergy and medications were performed before the challenges. Heart and lung auscultation, blood pressure, inspection of the oral cavity and skin were made before and during the challenges.

The open oral challenge tests to wheat were performed using wheat bread (Pågens, Sweden) with well-defined wheat content of 0.1/1 g wheat protein/g bread. The bread was given in increasing doses every 30 minutes in 5 or 7 steps from 0.005 g to 1.7 g of wheat protein. The maximum cumulative dose was 3.38 g of wheat protein (one slice of bread). Objective symptoms developing within two hours, which were scored according to the criteria of Astier (110) (Table3), were considered as a positive challenge. The challenges were stopped upon appearance of objective symptoms before the last dose was reached. A negative challenge was defined as no objective allergic symptoms within two hours after the last dose or no gastrointestinal symptoms 24 hours after the challenge.

Three children had experienced anaphylactic reactions within six months from the study visit, and three additional children refused challenge because of anxiety after experiencing severe reactions within the past year. These six children were not challenged for ethical reasons but are in the analyses considered to be wheat allergic, but are omitted in the analyses of severity of reaction.

Symptom score	Symptoms
0	**no symptoms**
1	abdominal pain that resolved without medical treatment, rhino conjunctivitis or urticaria <10 paplers, rash
2	**one organ involved** *abdominal pain requiring treatment *generalized urticaria *non laryngeal angioodema *mild asthma (cough, fall of peak expiratory flow <20%)
3	**two organs involved** (of symptoms mentioned under 2)
4	**three organs involved** (of symptoms mentioned under 2) **or asthma** requiring treatment or laryngeal oedema, or hypotension
5	**cardiac and respiratory symptoms requiring hospitalization in the intensive care unit**

Table 3. Symptom score according to Astier (110).

3.2.2 IgE-antibodies

The mix fx5 (Peanut, Cow milk, Egg white, Cod, Soybean, Wheat) and Phadiatop (birch, timothy, mugwort, mite, cat, dog, horse, mold) (ThermoFisher former Phadia AB, Uppsala, Sweden) was used for screening for common food and inhalant sensitizations at the first visit in the study. Before the challenge was performed blood samples were collected and stored in – 20 degrees pending analysis. IgE-ab were analysed for ImmunoCAP® (ThermoFisher former Phadia AB, Uppsala, Sweden) to whole wheat, recombinant ω-5 gliadin (Tri a 19) and a native gliadin preparation containing α-, β-, γ-, and ω-gliadins (gliadin), all available as commercial products. An IgE-ab level >0.35 kU_A/L was defined as positive. For Low molecular weight glutenin (LMW) and High molecular weight glutenin (HMW), experimental ImmunoCAP® tests were prepared with recombinant LMW-glutenin (Tri a 36) (111) and recombinant HMW-glutenin (Tri a 26) (49, 85). For HMW-glutenin the cut-off for a positive IgE-ab-level test was defined as 0.35 kU_A/L. This was in contrast to LMW-glutenin where the test was a research test with a high non-specific background binding and hence a cut-off of 1.5 kU_A/L was set based on the mean signal from 20 healthy blood donors + 3 SD.

IgE-ab were analysed with ImmunoCAP® (ThermoFisher, former Phadia AB, Uppsala, Sweden) for timothy, wheat, seven grass- specific components (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11 and Phl p12) and three wheat specific allergen components (Tri a 14, Tri a 19 and gliadin) as well as cross-reacting carbohydrate determinants (CCD) and Pru p 3 in children from the grass group. Six experimental tests with wheat specific recombinant allergens (two variants of Tri a 20 Tri a 36, Tri a 37, avenin-like protein and β-amylase) were also included in the panel. The cut-off for positive IgE-ab-levels in these experimental tests was set to ≥ 0.35 kUA/l, except for Tri a 36 which had a high non-specific background binding, and therefore the cut-off was set to ≥ 1.5 kUA/L based on the mean signal from 20 healthy blood donors + 3 SD.

3.2.3 Basophil allergen threshold sensitivity (CD-sens)

In the cohort described in paper II, blood samples were collected just prior to the challenge start in 13/24 children. In the remaining 11 cases blood samples were collected between one to ten months after the challenge test. The samples were stored at +4°C for a maximum of 24 hours before cell analyses were performed. Serum was separated and stored at -20°C pending analysis.
CD-sens evaluate allergen threshold sensitivity of basophils by using a dose response curve measuring percentage of activated basophils at different concentration of an allergen (112). Whole blood was incubated with RPMI (negative control) (Gibco Ltd., Paisley, Renfrewshire, U.K), N-formyl-methionyl-leucyl-phenylalanin (fMLP) (non-IgE-ab-dependent positive control) (Sigma Chemical Co, St. Louis, MO, USA), anti-FcεRI (IgE-ab-denendet positive control) (Bühlmann Laboratories, Basel, Switzerland) or allergen extracts of wheat (0.01-100 µg/mL) (IDD Thermo Fischer Scientific, Uppsala, Sweden), recombinant ω-5 gliadin (rTri a 19) (0.001-100n ng/mL) (IDD Thermo Fischer Scientific), Hydrolysed Wheat Protein (HWP) (0.01-100 µg/mL) (Meripro 711^{R}, Tate&Lyle, Aalst, Belgium) or timothy grass (0.1-100 µg/mL) (IDD Thermo Fischer Scientific, Uppsala, Sweden). Following this stimulatory step cells were immune stained with CD203c (Immunotech, Marseille, France) for basophil identification and CD63- (Immunotech) for detection of basophil activation. The cells were then analyzed in a Navios flow cytometer (Beckman Coulter, Inc., Fullerton, CA, USA). The cut-off value of 5 % of CD63-positive basophils was used to define a positive allergen test.

Basophils from patients which after anti-FcεRI stimulation (positive control) responded with <5% CD63-upregulation, were regarded as non-responders. For individuals with a response between 5-16 % (low-responders) the results should be interpreted with caution. The cut off 16 % was calculated (mean 76 % -3SD) from the positive controls of an in-house reference material of 264 allergic children and adults (90).

CD-sens was defined as the inverted value for the eliciting allergen concentration giving 50% (EC50) of maximum CD63% up-regulation multiplied by 100 [1/EC50×100] and was used to describe the patient's allergen sensitivity (113). A high CD-sens indicates a high basophil allergen threshold sensitivity (92).

3.2.4 Inhibition of IgE-ab binding

In paper III sera from patients displaying IgE-ab responses to both timothy and wheat was used to test for specific IgE-ab-binding inhibition experiments as previously described (Yman et al, 1975). In order to ensure trust worthy results of the analysis the level of IgE-ab to wheat needed to be >1 kUA/l and all but one sera meeting this criterion were selected (n=23). Timothy extract (14.4 mg/ml) in two dilutions, 1:1 and 1:1000 were used as liquid phase inhibitors. Inhibitor and patient serum, in equal volumes, were briefly mixed and incubated at room temperature for two hours. Thereafter IgE-ab measurement was done on the solid phase, wheat and timothy (control). The level of "no inhibition" was defined by mixing equal volumes of buffer and patient serum before a parallel incubation and IgE-ab measurement on the solid phase. Extracts of Dermatophagoides pteronyssinus and dog dander were used as control inhibitors, and did not cause inhibition of IgE-ab binding to neither timothy nor wheat.

Simultaneous IgE-ab analysis was also performed using CCD as solid phase, for two sera that had an initial IgE-ab level >1 kUA/l to CCD. Similarly, for three sera with an initial IgE-ab level to Phl p 12 >1 kUA/l, wheat extract (4.8 mg/ml) was used as liquid phase inhibitor in two dilutions, 1:1 and 1:1000, before analysis on the solid phase, Phl p 12 and wheat (control).

Inhibition was defined as the relative reduction of detected IgE-ab response between the two measurements:

$$\text{Inhibition (\%)} = \frac{\text{(IgE-ab binding for sera diluted in buffer – IgE-ab binding for sera diluted in inhibitor)}}{\text{(IgE-ab binding for sera diluted in buffer)}} \times 100$$

3.2.5 QoL questionnaires

Parents and patients (depending on age) in the wheat group were interviewed with use of four QoL questionnaires: the Child Health Questionnaire – Parental-completed form 28 (CHQ-PF28) (114) and the Child Health Questionnaire – Child-completed form 87 (CHQ-CF87) and Food Quality of Life Questionnaire – Parents Form (FQLQ-PF) and Food Quality of Life Questionnaire – Teenagers Form (FQLQ-TF) (105).

Parents and patients in the grass group were interviewed with a standardized questionnaire with focus on symptoms of grass pollen allergy, asthma, rhinocunjunktivitis, eczema, food allergies, and questions about outgrown food allergies and the same questionnaires as the wheat group

The questionnaires used were in Swedish validated and tested for reliability and translated to Swedish back and forth following international guidelines by Norrby et al (115).

Both CHQ-PF28 and the CHQ-CF87 had items with 4, 5 or 6 response options, divided over 10 multi-items (Table 2, Paper IV). The items were summed up (some recoded/recalibrated) and for ease of the interpretation the items were transformed into 0 (lowest score) to 100 (highest score), with higher score indicating better well-being.

All questions were based on a retrospective recall of health over the preceding 4 weeks, except for one single item that measures change in health over the preceding year.

3.3 STATISTICAL ANALYSIS

The statistical analyses were performed using Prism 5, Graph Pad Software, La Jolla, USA (Paper I, II and III) and IBM SPSS 22.0, Chicago, ILL, USA (Paper IV). The material from the two Study populations was not normally distributed and non-parametric statistical methods were used. No adjustment for multiple testing has been performed. Thus, significant results should be regarded as descriptive and explorative.

3.3.1 Fisher's exact test (I, III)

Fisher's exact test was used for pairwise comparison of categorical data of clinical characteristics in patient with wheat allergy challenge positive respectively negative in paper I and in children with grass pollen allergy sensitized or not sensitized to wheat in paper III. A p-value <0.05 was considered significant, ns= not significant.

3.3.2 Mann-Whitney U-test (I-IV)

Mann-Whitney U-test was used for comparison of IgE-ab levels to wheat and different wheat components in paper I (wheat, timothy, ω-5 gliadin, gliadin, HMW/LMW- glutenin).

A p-value of <0.05 was considered significant. Specific IgE-ab levels ≤0.35 kUA/L were set to 0.175 and for LMW-glutenin antibody levels ≤ 1.5 kUA/L were set to 0.75 for statistical analyses.

In paper II the test was used to assess differences in between the children with wheat allergy positive and negative challenges. Significance was considered at a p-value of <0.05.

In paper III the same test was used for comparison of IgE-ab levels to wheat and timothy. A p-value of <0.05 was considered significant. IgE ab levels under the cut-off were set to 0.05 for statistical analyses.

In Paper IV, differences in demography and characteristics of children diagnosed with grass respectively wheat allergy were examined and geometric means and standard deviations (SD) for the different item scores were calculated and differences in distribution of parent /children reported geometric mean scores between the two groups (wheat and grass allergic patients) were assessed using Mann-Whitney U. To adjust for potential confounders, including age (continuous), sex, asthma (yes/no), and pollen season (yes/no), we used a general linear model to assess beta coefficients (B) and 95% confidence intervals (CI) to describe the differences in CHQ-PF28 and CHQ-CF87 mean scores in wheat allergic subjects compared to grass allergic subjects.

3.3.3 ROC curves (I)

ROC curves were calculated for ImmunoCAP specific IgE-ab for wheat and the four wheat components (ω-5 gliadin, gliadin, HMW/LMW- glutenin), with the food challenge results as reference and reported as the area under the curve (AUC). The diagnostic performance, in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), for each test was calculated for a cut-off level of 0.35 kUA/L for wheat, ω-5 gliadin, gliadin and HMW-gliadin and for a cut-off level of 1.5 kU_A/L for LMW-glutenin. The cut-off level producing a specificity of at least 90% and 95% (here defined as the positive decision points), and finally the cut-off level producing a sensitivity of at least 90% and 95% (here defined as the negative decision points) was calculated for all tests.

3.3.4 Spearman rank order correlation (r_s) (II, IV)

This test was used in paper II to assess the relation between the wheat challenge, CD-sens, wheat, omega 5 gliadin, hydrolyzed wheat and IgE-ab measurements.

Spearman rank order correlation (r_s) coefficients were used in paper IV between scores for similar items on the parent reported CHQ-PF28 and the child reported CHQ-CF87 for patients with wheat and grass allergy. Furthermore it was used to assess the relation between the wheat challenge, CD-sens wheat, omega 5 gliadin, hydrolyzed wheat and IgE-ab measurements.

3.3.5 Chi-square

Test was used in paper IV for categorical variables, differences in demography and characteristics of children diagnosed with grass respectively wheat allergy.

3.4 ETHICAL APPROVAL

The studies in the thesis were approved Regional Ethical Review Board at Karolinska Institutet in Stockholm, Sweden (Identification number: 2008/562-31/3 and 2011/1833 32). Written informed consent was obtained from all study objects and parents before the children were included in the study.

4 RESULTS

4.1 PAPER I

Paper I demonstrates that CRD are useful and may improve diagnosis of wheat allergy.

4.1.1 Oral food challenges with wheat

We found 102 children who were sensitized to wheat, had a doctor´s diagnosis of wheat allergy and eliminated products containing wheat from their diet and seventy six children, aged 1-17 years, fulfilled the inclusion criteria (Paper I).

However, thirteen children were excluded during the study; four children did not fully participate, three were negative in IgE-ab to wheat at the inclusion, in three children blood samples could not be obtained , two children developed an autoimmune disease and one child did not complete the wheat challenge (Paper I, II and IV).

Sixty-three patients with doctor's diagnosis of wheat allergy were included in the study. Blood samples were taken before the challenges and a clinical examination was performed (visit 1).

An open oral challenge (OFC) with wheat was performed for 57 children after clinical examination (visit 2). Six children did not go through an OFC due to history of severe reactions to wheat during last year.

Thirty-two children (51%) had a positive reaction at the challenge, the wheat allergic group (WA) and 31 children had a negative challenge test result, non-wheat allergic group (non-WA) (Table 4).

There was no significant difference regarding medical history or age (6.5 respectively 4 years) between the WA and the non-WA (Table 4).

Furthermore there was no significant difference in the prevalence of other allergic diseases, e.g. symptoms of grass pollen allergy were found to be equally common in both groups.

Noteworthy, the levels of IgE-ab to grass pollen was higher in the WA group, median (range) 5.1 kUA/l (<0.35 – 49) than in the non-WA group 0.75 kUA/l (<0.35 – 85).

The most common allergic reactions during the two hours of observation time at the challenge were respiratory symptoms (n=10) followed by rhino-conjunctivitis, angioedema, rash and oral allergy syndrome (OAS) (n=8) and other symptoms, urticaria respectively gastro-intestinal symptoms (n=7).

Out of the 31 non-WA children, only four developed symptoms at the follow-up 72 hours after challenge (two gastrointestinal symptoms, two worsening of their eczema and one developed urticaria). None of the symptoms were regarded as severe according to the parents. Therefore the advice to continue re-introduction of wheat into the child's diet was not changed.

Patient characteristics	All	Final wheat allergy diagnosis		p-value
		Non-WA	WA	
Total number	63	31	32	ns
Sex, male/female	41/22	19/12	22/10	ns
Age, yr; median (range)	5.0 (1–17)	4.0 (1–17)	6.5 (1–17)	ns
Reported allergies; number (%)				
Asthma	41 (65)	17 (55)	24 (75)	ns
Rhino-conjunctivitis	35 (56)	17 (55)	18 (56)	ns
Eczema	40 (63)	18 (58)	22 (69)	ns
Any pollen allergy	43 (68)	22 (71)	21 (66)	ns
Grass pollen allergy	25 (40)	12 (39)	13 (41)	ns
Furry animal allergy	29 (46)	13 (42)	16 (50)	ns
Other food allergy	55 (87)	27 (87)	28 (88)	ns
Egg	49 (78)	24 (77)	25 (78)	ns
Milk	45 (71)	22 (71)	23 (72)	ns
Fish	10 (16)	6 (19)	4 (12)	ns
Peanut/tree nuts	33 (52)	13 (42)	20 (62)	

Table 4. Demographic data and clinical characteristics of all study subjects and in subjects confirmed as wheat allergic (WA) or non-wheat allergic (non-WA) based on challenge outcome (n = 57) convincing history of anaphylaxis (n = 6).

4.1.2 Comparisons of wheat-allergic and non-wheat-allergic subjects

The levels of IgE-ab s to each of the four studied components (ω-5 gliadin, HMW-glutenin, LMW-glutenin and gliadin) and to wheat was significantly higher in WA children compared to non-WA ($p < 0.001$) (Fig 10). All six children that were considered to be wheat allergic but that were not challenged had detectable IgE-ab to all four wheat components.

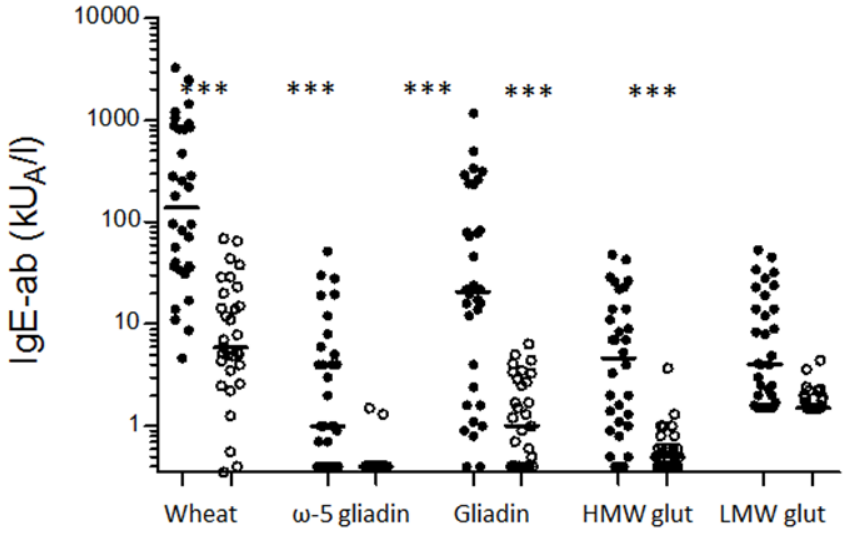

Fig 10. IgE-ab levels to wheat and wheat components in 32 children with WA (●) and 31 non-WA (○) children. Statistical significance was calculated based on the presence or absence of IgE-ab above the set thresholds for each test (refers to a level of significance <0.001).***

Receiver operating characteristic (ROC) curves was calculated for all components and showed that IgE-ab to HMW-glutenin had the largest area under the curve (AUC) 0.88, while AUCs varied between 0.78 and 0.82 for the other wheat components tested (Table 5). At the respective assay cut off points for each test, ω-5 gliadin had a specificity of 84 % and a sensitivity of 62%, while gliadin, HMW-glutenin, and LMW-glutenin had sensitivities of 81–94 % and specificities between 29 and 52 % (Table 5). The positive decision points based on at least 95 % clinical specificity in the diagnosis of wheat allergy were 70 kUA/l for wheat, 1.3 kUA/l for ω-5 gliadin, 6.0 kUA/l for gliadin, 1.4 kUA/l for HMW-glutenin, and 4.0 kUA/l for LMW-glutenin (Table 5). Negative decision points, defined as a sensitivity of at least 95%, were only possible to calculate for wheat (8 kUA/l) and HMW-glutenin (0.35 kUA/l) (Table 5).

Diagnostic performance	**Wheat allergens**				
	Wheat (f4)	**ω-5 gliadin**	**Gliadin**	**HMW-glutenin**	**LMW-glutenin**
ROC/AUC	0.91	0.78	0.83	0.88	0.82
Assay cut-off point					
Specific IgE-ab level (kU_A/l)	0.35	0.35	0.35	0.35	1.5
Sensitivity, Specificity (%)	100, 6	62, 84	94, 29	97, 42	81, 52
PPV, NPV (%)	52, 100	80, 68	58, 82	63, 93	63, 73
Positive decision point (95% spec)					
Specific IgE-ab level (kU_A/l)	70	1.3	6.0	1.4	4.0
Sensitivity, Specificity (%)	62, 97	44, 97	69, 97	66, 97	56, 97
PPV, NPV (%)	95, 71	93, 62	96, 75	95, 73	95. 68
Negative decision point (95% sens)					
Specific IgE-ab level (kU_A/l)	8.0	None	None	0.35	None
Sensitivity, Specificity (%)	97, 58			97, 42	
PPV, NPV (%)	70, 95			63, 93	

Table 5. ROC/AUC calculations for wheat components.

4.1.3 Relation between symptom score and IgE-ab levels to wheat components

For the children that reacted positively at challenge, the severity of reactions was graded according to Astier (Table 3) and resulted in a median score of 2.5 on a five-point graded scale. Thirteen children were graded as score 1, four score 2, and nine score 4. No child was classified as having scores 3 or 5. A higher score indicates a more severe reaction.

The challenge positive children (n = 26) were divided into two severity groups (mild = severity score 1 and 2; severe = score 4), and the IgE-ab levels to wheat components were compared to those of non-WA children (Fig 11). Both severity groups had significantly higher levels of IgE-ab to all four components as compared to non-WA (p between >0.05 and >0.001), apart from gliadin where there was no difference between mild reacting children and non-WA. Children with severe symptoms compared to those with mild symptoms had significantly higher IgE-ab levels to gliadin, HMW-glutenin and LMW-glutenin (p > 0.05) but not to ω-5 gliadin.

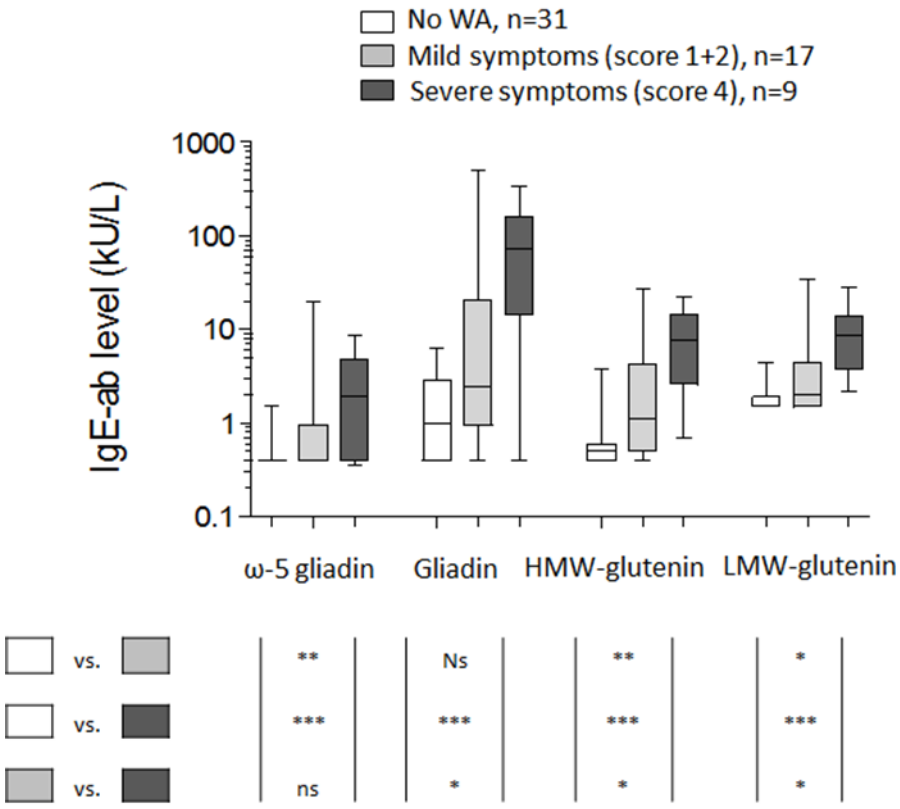

Fig 11. IgE-ab levels to four gluten-derived wheat components in 31 non-WA and 26 children positive in wheat challenge divided into groups with mild and severe symptoms, respectively. Results are presented as the 25th to 75th percentile of the values with the median and min to max indicated. Statistical significant differences between the groups are indicated (*refers to a level of significance <0.05, **<0.01 and ***<0.001).

4.2 PAPER II

Paper II showed that combining CD-sens and IgE-ab to wheat or wheat components may be useful in the diagnosis and follow-up of wheat-allergic children.

4.2.1 Challenge Outcome

Of the 24 patients included in the study, 12 (50 %) had a positive reaction at the oral wheat challenge (table 6). The median age of those with a positive challenge test was 7 (2-15) years and for children with a negative challenge it was 4 (2-13) years.

The outcome of the challenge was only reported as positive or negative (table 6) even though a scoring of all symptoms was done. Of the 12 patients with a positive challenge, 8 had mild-to-moderate symptoms (grade 1-3) and 4 patients experienced severe symptoms (grade 4).

The challenge positive children had significantly higher concentrations of IgE-ab to wheat ($p < 0.01$), to ω-5 gliadin ($p < 0.005$) and to HWP ($p < 0.005$) compared to children with a negative outcome at the challenge. There was no significant difference between IgE-ab levels to timothy in the challenge-positive as compared to the challenge-negative group.

All patients in paper II ($n = 24$) were tested for CD-sens to wheat, ω-5 gliadin and timothy. All 21 patients had a positive CD-sens to wheat and there was a tendency ($p = 0.08$) for the wheat CD-sens value to discriminate between children positive or negative in wheat challenge. Children with a positive challenge had higher levels of CD-sens.

Six of 11 children with a positive challenge also had a positive CD-sens to ω-5 gliadin.CD-sens for HWP was performed only in 20/24 patients due to scarcity of material and was possible to evaluate in 17 patients. Eleven of those 17 patients had a positive CD-sens reaction to HWP, and 7 of these 11 were positive in wheat challenge.

		IgE-ab (kU_A/L)				CD-sens			
Patient	**Wheat challenge**	**Wheat**	**ω5-gliadin**	**HWP**	**Timothy**	**Wheat**	**ω5-gliadin***	**HWP**	**Timothy**
1	+	37	0.18	20.2	0.59	1659	+	n.t.	150
2	+	11	0	6.0	2.0	303	-	n.t.	6.9
3	+	3.7	0.27	0.47	6.4	5.0	+	0	71.7
4	+	2500	14	160.4	20	1305	+	191	3.5
5	+	255	4.3	108.4	6.6	9896	+	814	169
6	+	40	0.62	13.6	49	1678	-	49.6	583
7	+	35	0.34	14.6	0.43	1046	-	11.8	1.87
8	+	22	0.12	8.2	0.36	515	+	75	+
9	+	93	0.13	26.6	0	n.r.	n.r.	n.r.	n.r.
10	+	940	4.1	47.3	0.17	+	-	0	0
11	+	72	0.29	28.9	1.0	5326	+	13	7.56
12	+	57	0	10.8	13	177	-	23.7	4.26
13	-	14	0	2.8	0.97	1145	-	32.4	8.2
14	-	5.3	0.11	1.3	0	319	-	0	0
15	-	7.8	0	0	5.9	12.7	-	0	345
16	-	6.7	0	2.1	0	621	-	8.32	1.98
17	-	1.3	0	0.2	0.96	n.r.	n.r.	n.r.	n.r.
18	-	69	0.17	3.8	4.5	55.5	-	3.05	3.18
19	-	31.8	0.05	4.0	0	62.1	+	0	0
20	-	6.6	0	5.9	1.6	17.3	-	0	1.94
21	-	74	0	37.3	3.0	1427	-	23.9	3.1
22	-	18	0	3.0	0.12	514	-	n.t.	0
23	-	2.9	0.21	0.41	0.73	51.7	-	n.t.	0
24	-	0.6	0	0.15	0	l.r.	l.r.	l.r.	l.r.

n.t. = not tested; n.r. = non responder; l.r = low responder; + = positive; - = negative

Table 6. Serological and cellular results.

4.2.2 Challenge outcome in relation to combination of IgE-ab and CD-sens

There was a significant correlation between the levels of IgE-ab and the CD-sens values to wheat ($r = 0.64$, $p < 0.003$; fig. 12 a). If a wheat CD-sens value >150 was combined with a wheat IgE-ab concentration >20 kU_A/l, or IgE-ab to ω-5 gliadin >0.1 kU_A/l, 83% of the wheat challenge outcomes were predicted. If the non- and low responders were excluded, the agreement was 91%.

There was also a significant correlation between the level of IgE-ab to HWP and the CD-sens to HWP ($r = 0.59$, $p < 0.05$; fig. 12 b). All challenge-positive children, except 2 non-responders, had a CD-sens value to HWP >10 and an IgE-ab level to HWP >8 kU_A/l. Five children had IgE-ab to HWP but were negative in CD-sens to HWP. Of these 5 children, 3 were also negative in the wheat challenge (fig 12c).

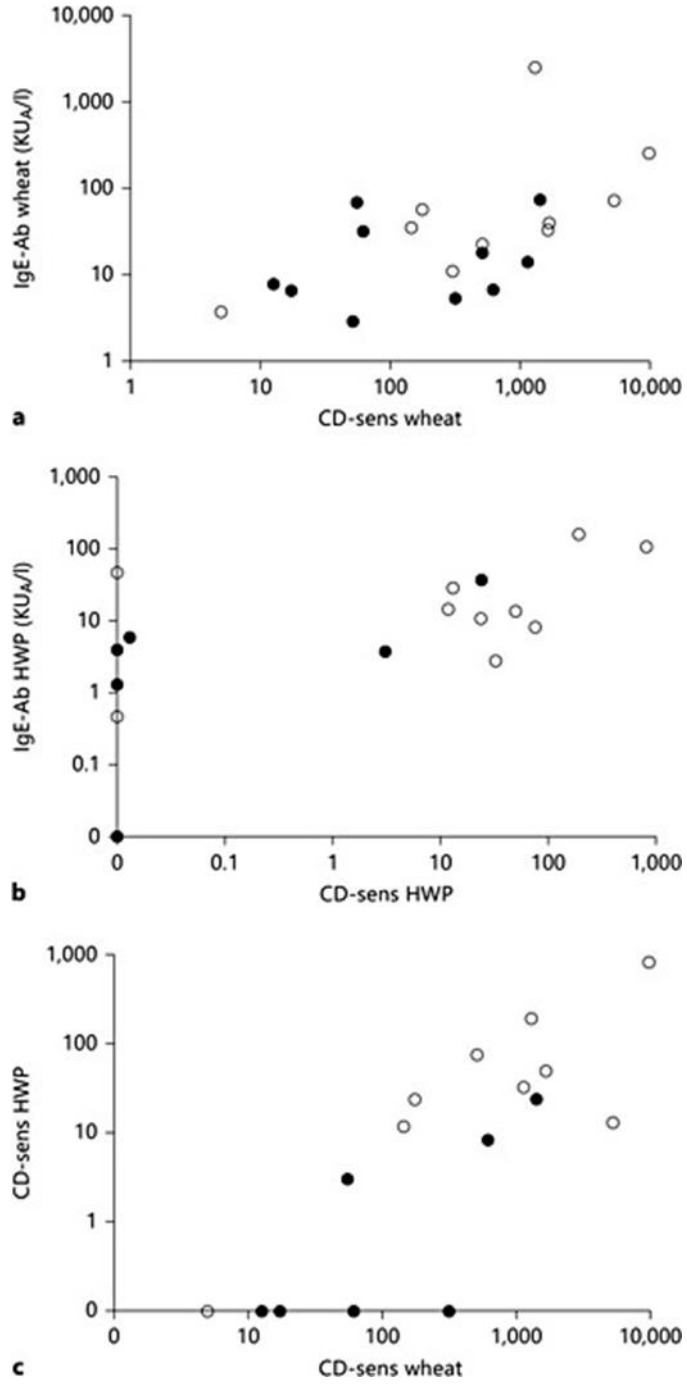

Fig 12. IgE-ab in relation to basophil allergen threshold sensitivity, CD-sens, for wheat (a) and HWP (b). (c) CD-sens to HWP in relation to CD-sens to wheat. ● = Positive challenge; ○= negative challenge.

4.3 PAPER III

Paper III demonstrates that the majority of grass allergic individuals had IgE-ab to wheat, however at low concentrations. This is interpreted as cross reactivity between grass pollen and wheat and could in one third of the patients be explained by sensitisation to profilins and CCD.

4.3.1 Sensitization to grass, wheat and related components

Seventy-four children were included in the study but two children were excluded when they neither had clinical symptoms nor IgE-ab for grass (Paper III and IV). Forty-seven (65 %) were boys and the median age was 12 (5-17) years. Sensitization to wheat was confirmed in 43 (60 %) of the children (Fig 13). Another four children had a history of wheat allergy but they had developed tolerance. Comparing the wheat sensitized children (n=43) to the non-wheat sensitized, timothy allergic children, there was no differences regarding reported asthma, rhino-conjunctivitis, eczema and food allergy. The medical records for the patients showed that egg, milk, soy, fish and nuts/peanuts were the major allergens causing both past and current allergies.

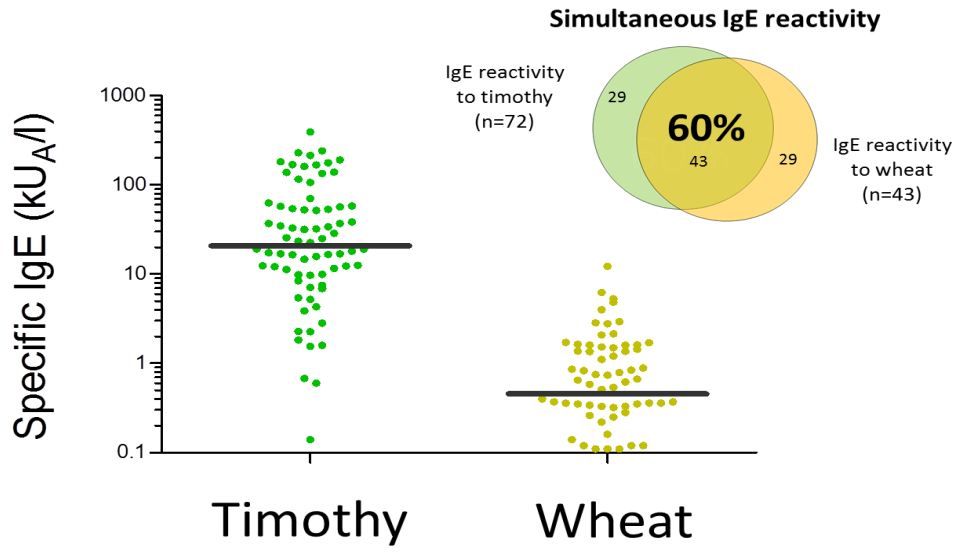

Fig 13. IgE reactivity to timothy and wheat in 72 children with grasspollen allery.All children displayed IgE-ab-ab to timothy (median level 20 kU_A/l) and out of these 44 children were sensitized to wheat (median level 1,2 kU_A/l)

The median level of IgE-ab response to wheat in sera from the 72 children was 1.2 kU_A/l with a maximum level of 12.2 kU_A/l . The IgE-ab response to timothy was significantly higher than that to wheat ($p<0.001$). Furthermore the IgE-ab responses to seven different wheat specific allergen components were also measured. The most frequent IgE-ab response was obtained to the wheat LTP component Tri a 14 (15 %) followed by Gliadin, Tri a 36, and avenin-like protein (all 4 %), and Tri a 19, Tri a 20_{long}, Tri a 37 and β-amylase (all 3 %), and Tri a 20_{short} (1 %). The level of IgE-ab responses to wheat components were generally low, <1.5 kU_A/l.

Twenty-three sera with an IgE-ab response >1 kU_A/l to wheat were inhibited with timothy extract and the degree of inhibition varied between 11-94%, with a median inhibition degree of 66 % with timothy extract diluted 1:1

4.3.2 Inhibition of specific IgE responses to CCD and profilin

Of the children included in the study five out of 72 sera displayed IgE reactivity to CCD and the response ranged between 0.4-7.8 kU_A/l. All sera also displayed a simultaneous IgE-ab response to wheat. Inhibition of two sera, both with an IgE-ab to CCD and wheat >1 kU_A/l, with timothy extract reduced the binding to CCD and wheat by 94-98% and 86-92%, respectively. Sera from 14 children displayed IgE-ab to timothy, profilin and Phl p 12 and out of these 12 children had a simultaneous reactivity to wheat. Further, two sera also displayed simultaneous reactivity to CCD and wheat. Inhibition of five sera, with an IgE-ab response to both Phl p 12 and wheat >1 kU_A/l, with wheat extract reduced the binding to Phl p 12 and timothy by 19-90% and 7-20%, respectively (Table 7).

Simultaneous IgE reactivity n(%)			
Timothy Wheat	**Timothy Wheat Profilin**	**Timothy Wheat CCD**	**Timothy Wheat CCD Profilin**
43(60%)	12(17%)	5(7%)	2(3%)

Table 7. Number of children displaying simultaneous IgE reactivity to timothy, wheat, CCD and profilin.

4.4 PAPER IV

Paper IV demonstrates that wheat allergy impairs health-related-quality-of-life more than grass allergy in childhood.

4.4.1 Parental CHQ-PF28 questionnaire

Fifty-five parents of the 63 wheat allergic children filled out the CHQ-PF28 questionnaire (Table 8). The domain with the lowest score was Parental impact-Time (61.4) whilst the domain Role/Social limitations-Emotional- Behavioural received the highest score (87.4).

Of the grass allergic patients 68 out of 72 answered the questionnaire (Table 8). For this group the Change in the Health domain received the lowest score (67.3) and Role/social limitations-Emotional- behavioural received the highest score (94.1). There was a significant difference between the parent's perception and their wheat allergic children for five items compared to parent's perception and their grass allergic children. These items were *general behaviour* ($p<0.0001$), *general health perceptions* ($p=0.001$), parental impact of *emotions* ($p=0.001$), and *time* ($p<0.0001$), as well as *family activities* ($p=0.001$). Interestingly, the opposite was true for the item Change in Health. The parents of the grass allergic group scored this item significantly lower ($p=0.020$) compared to the wheat group's parents.

Items	Wheat allergic (n=55) geometric mean	SD	Grass allergic (n=68) geometric mean	SD	P-value*
Physical functioning	86.5	18.3	90.1	11.0	0.350
Role/social limitations-Emotional-behavioural	87.4	20.3	94.1	13.1	0.323
Role functioning: Physical	85.9	19.4	91.8	15.3	0.119
Bodily pain	81.0	18.9	77.4	22.7	0.956
General behaviour	61.8	11.6	75.7	15.1	<0.0001
Mental health	77.5	14.2	78.5	15.2	0.520
Self esteem	82.2	13.7	84.3	12.8	0.478
General health perceptions	81.7	18.8	91.8	18.6	0.001
Change in health	74.3	18.0	67.3	18.5	0.020
Parental impact-Emotional	70.2	23,0	86.3	14.3	0.001
Parental impact-Time	61.4	18.3	73.9	10.4	<0.0001
Family activities	72.7	22.9	88.8	14.3	0.001
Family cohesion	66.1	20.7	71.7	20.6	0.195

* Differences in distributions of scores across groups tested using Mann-Whitney U-test.

Table 8. Parent-reported geometric mean scores (standard deviation, SD) for the CHQ-PF28 among children with wheat allergy and grass allergy respectively. Score range from lowest score 0 and highest score 100.

4.4.2 Children CHQ-CF87 questionnaire

Of the wheat allergic children 27 out of 63 were able to read and fill out the CHQ-CF97 questionnaire (Table 9). Mental Health received the lowest score (68.5) and the item that with the highest score (99.7) was Role functioning: Behaviour.

Amongst the grass allergic, 51 out of 72 children filled out the questionnaire (Table 9). A similar pattern was seen for the grass allergic group. Again it was Mental Health (66.0) that received the lowest score and the item that received the highest score (95.5) was Role functioning: Behaviour.

The grass allergic children reported significantly lower scores for four items compared to the wheat allergic children. These are Physical functioning (p=0.008), Role functioning; Emotions (p=0.005), Role functioning: Behaviour (p=0.029) and Self-esteem - overall subjective emotional evaluation (p=0.035). The wheat allergic children did not score lower than the grass allergic children in any of the recorded items.

Items	Wheat allergic (n=27) geometric mean	SD	Grass allergic (n=51) geometric mean	SD	P-value*
Physical functioning	96.1	7.9	93.0	8.2	0.008
Role functioning: Emotional	99.0	2.7	90.4	13.8	0.005
Role functioning: Behaviour	99.7	1.6	95.5	8.7	0.029
Role functioning: Physical	96.6	7.4	91.8	12.5	0.167
Bodily pain	83.9	13.5	77.9	18.1	0.250
General behaviour	81.9	13.7	82.5	9.8	0.468
Mental health	68.5	5.4	66.0	6.4	0.179
Self esteem	88.1	10.6	82.1	11.4	0.035
General health perceptions	74.2	12.5	73.7	13.7	0.832
Change in health	64.2	17.8	67.8	21.1	0.324
Family activities	87.2	10.8	86.3	12.4	0.941
Family cohesion	69.8	20.1	66.5	22.2	0.899

* *Differences in distributions of scores across groups tested using Mann-Whitney U-test.*

Table 9. Child-reported geometric mean scores (standard deviation, SD) for the CHQ-CF87 among children with wheat allergy and grass allergy. Score range from lowest score 0 and highest score 100.

5 DISCUSSION

This thesis focus on wheat allergy and the aim was to evaluate the characteristics of patients IgE sensitized to wheat, both clinically and immunologically through wheat challenges, component resolved diagnostics (CRD), basophil stimulation in vitro (CD-sens) and quality of life (QoL).

5.1 ORAL WHEAT CHALLENGES

The only available procedure to confirm whether a patient is truly allergic to food or not, is to perform an oral food challenge test.

The preferred method is the double-blind placebo-controlled food challenge (DBPCFC), both in the clinic as well as in research. There are a number of guidelines for how to perform a DBPCFC (94, 97). The advantage with DBPCFC is that it reduces the possible anxiety related reactions compared to an open oral food challenge test (OFC). The disadvantage is that DBPCFC is time-consuming and more complicated to perform. The reason for performing OFC in our study was to optimize the recruitment and compliance of our study patients. Some parents were not willing to participate in two challenges and we were eager to have as representative sample of wheat allergic individuals as possible. We can only speculate if we would have received different results if we would have used DBPCFC instead of OFC. Four children reacted with mild late onset symptoms, after the challenge, when they had returned home. These symptoms would have been easier to interpret if DBPCFC had been used from a clinical point of view. However, this did not change the study results as we only included immediate type symptoms during the challenge and up to two hours after challenge.

Half of the children with a doctor´s diagnose of wheat allergy in our study did not react when challenged to wheat indicating an over-diagnosis of wheat allergy. However, some of the children might have been wheat allergic but have grown out of the disease but they have not been evaluated. This emphasises the need to follow up children with wheat allergy regularly and to perform challenges in daily practise. This is important especially in patients on a wheat free diet where the clinical history is not aligned with serological tests. Performing a wheat challenge is even more important when CRD is not available. Our data has recently been confirmed by Winberg et al in their population based cohort Study in northern Sweden (116). They show there was a high discrepancy in the prevalence of allergy to cow´s milk, hen´s egg, cod and/or wheat as estimated by reported data versus determination by clinical evaluation or DBPCFC.

The reason for not reacting to wheat in our study is most probably due to tolerance development or to over-diagnosis caused by a cross-reaction between wheat and allergens of other origin such as cross-reacting carbohydrate determinants and/or profilins from pollen. It is therefore important to continuously evaluate a child with a wheat allergy diagnosis serologically. If the IgE-ab to wheat and its relevant components indicate tolerance development, then a challenge should be performed as soon as possible.

5.2 USEFULNESS OF CRD AND CD-SENS IN WHEAT ALLERGIC PATIENTS

5.2.1 CRD to wheat components

We investigated the diagnostic properties of wheat component IgE-ab compared to OFC to wheat. The levels of IgE-ab to wheat gluten-derived components gliadin, ω-5 gliadin, HMW-glutenin and LMW-glutenin correlated well with the OFC outcome and severity. Children with a confirmed WA had significantly higher IgE-ab levels to gluten-derived wheat components compared to the challenge negative group non-WA. Furthermore the severity of the reactions at the challenge correlated with the IgE-ab levels to these wheat

components. The more severe reactions the higher the levels of IgE-ab to the gluten-derived components. The IgE-ab levels were also very high in children with a convincing recent history of wheat allergy.

In our study the wheat extract test had high sensitivity which, at least in part could be explained by the inclusion criteria of positive wheat IgE tests. However the specificity was very low, illustrating the need for more specific diagnostic tools, as suggested by others (5, 9).

The ω-5 gliadin was the component showing the highest specificity and was best in discriminating between children with WA from non-WA children. This finding is supported by previous reports in the literature where gliadins have been shown to be useful in SPT in the diagnosis of WA children (39). The sensitivity of IgE-ab to ω-5 gliadin in our study was rather low (62%), and one-third of the WA children were not sensitized to ω-5 gliadin. However, the eleven children without ω-5 gliadin IgE-ab were all sensitized to one or more of the other three gluten-specific allergen components (HMW/LMW-glutenin and gliadin). Others have reported that wheat-allergic patients more frequently have IgE-ab to LMW-glutenin than to ω-5 gliadin (84). Even so not all wheat-allergic patients could be identified by combining IgE-ab against LMW-glutenin and ω-5 gliadin in that study. Simonato et al (77) found a relation between the presence of IgE-ab to LMW-glutenin and gastrointestinal symptoms after wheat ingestion. The results from our study indicate that also in WA with immediate reactions, IgE-ab to a combination of gluten-derived component improves the prediction of clinical symptoms.

Hydrolyzed wheat protein (HWP) is often used in food or cosmetics and it can cause the same reactions as gluten when ingested. In many cases, HWP is a hidden allergen due to its emulsifying function and might easily not be detected as a wheat allergen. Of the 24 children in paper ll, all were positive to IgE-ab to wheat, 96 % also had IgE-ab to HWP. This conforms with the theory suggested by Leduc et al (117) that human subjects appear to be easily sensitized to HWP. One possible explanation is that since HWP is not a natural protein, human subjects have not had sufficient time to develop tolerance compared to regular staple foods. We suggest that the patients with a strict wheat-free diet also should take precautions to avoid HWP.

5.2.2 CD-sens to wheat and some wheat components

CD-sens were analysed in a subpopulation of 24 children and the purpose was to show whether basophils stimulated with wheat, ω-5 gliadin and HWP or a combination of CD-sens and IgE-ab to wheat and/or ω-5 gliadin could enhance the prediction of the wheat challenge outcome (Paper II).

Neither CD-sens or IgE-ab to ω-5 gliadin alone provided a satisfactory prediction of the challenge outcome hence we combined the different markers and a combination of CD-sens to wheat >150 together with IgE-ab to wheat >20 kUA/l or IgE-ab to ω-5 gliadin >0.1 kUA/l gave a prediction of 83% of the challenge outcome. Compared with Ebisawa et al (81) this represents a diagnostic improvement for recombinant ω-5 gliadin based on challenge in Japanese children. Based on our data, we can speculate that the basophils only in the severe wheat-allergic patients react to HWP and that this hypersensitivity is not a separate entity as suggested by Chinuki et al (118).

Tokuda et al (119) reported that basophil response to ω-5 gliadin predicted wheat allergy in children, a finding we cannot confirm based on our material. In our study we used recombinant ω-5 gliadin whilst Tokuda et al used both purified native ω-5 gliadin and recombinant ω-5 gliadin. They found the native form of ω-5 gliadin to discriminate better than the recombinant form which could explain the difference between the two studies.

The children who did not react to the wheat challenge non-WA, were younger, and had lower, but detectable, CD-sens values and lower concentrations of IgE-ab to wheat compared to WA. Thus we speculated that IgE-ab-sensitized basophils precede target organ sensitivity and thus might indicate later development of clinical symptoms of food allergy as well as future airway allergy. This was in contradiction by the findings from paper lll where half or our timothy allergic children were IgE sensitised to wheat but tolerated wheat. Four of them reported a previous wheat allergy.

It is well known that sensitisation to wheat is far more common than true wheat allergy (5, 17) (60). To diagnose wheat allergy is often complicated by a concurrent pollen allergy leading to cross reactions timothy and to false-positive wheat extract test results. It is often unclear whether the wheat sensitization reflects a specific IgE-ab-response to wheat or a potential cross-reactivity with pollen allergens. Thus, there is a need for more specific wheat allergy biomarkers. It is also unclear how common sensitization to wheat is among timothy pollen allergic individuals. In paper l we compared children with wheat allergy to those who were not wheat allergic and found that the number of children with symptoms of timothy pollen allergy was equal. Interestingly, we found significantly higher levels of IgE-ab to timothy pollen in the WA group compared to non-WA, even though there was no difference in the sensitization rate of timothy in the two wheat groups. These children also had higher IgE-ab levels to wheat extract; indicating that sensitization to timothy pollen influences the apparent IgE-ab level to wheat. A previous study has shown that 65% of the patients with timothy pollen allergy had false-positive IgE-ab test results to wheat extracts (103). In our paper ll we utilised CD-sens and we saw similar results since most of the children with IgE-ab to wheat also had IgE-ab to timothy and that they had a positive CD-sens to the same allergens. To our knowledge, this has not been shown before and is in contrast to findings of Jones et al (8) that showed little in vitro cross-reactivity to timothy among WA patients. One of our most interesting findings is that 65% of the children that had IgE-ab and a positive CD-sens to timothy had no symptoms to timothy pollen. There is thus a need for markers that truly can differentiate between primary wheat sensitization and cross-reactive sensitization.

5.3 CROSS-REACTION BETWEEN CHILDREN IGE SENSITIZED TO TIMOTHY AND WHEAT

5.3.1 CRD to wheat and timothy

We investigated children IgE sensitised and allergic to timothy of whom all tolerated wheat, for IgE-ab to wheat and timothy components and compared them to the children with a doctor´s diagnose of wheat allergy (Paper III).

Almost two thirds of the timothy allergic children had IgE-ab to wheat supporting serological data from other studies (104, 120). Sensitization to both wheat and timothy may be explained by a serological relationship between the two allergens, i.e. cross-reactivity. Simultaneous sensitization could also be due to co-sensitization to the two allergens or a combination of co-sensitization and cross-reactivity.

When studying the frequencies and levels of IgE-ab to wheat specific components, among the timothy allergic children, low levels were found in most children. Almost all responders were found in the group of children with dual sensitization to timothy pollen and the whole wheat extract. The highest frequency of IgE-ab responses was to wheat LTP (Tri a 14), and it was significantly higher in the wheat sensitized children. Tri a 14 is a cross-reactive allergen found in many foods, trees and weeds but not in timothy and has also been shown to be associated with symptoms when eating wheat (111, 121-123). Possible explanations for the IgE-ab responses to Tri a 14 found in these children currently eating wheat could be the presence of remaining IgE-ab from an earlier sensitization connected with wheat allergy, cross-reactivity from other cereals or a wheat sensitization without current symptoms. Interestingly, only four of the timothy pollen allergic children had experienced wheat allergy in the past. A wheat specific allergen associated with symptoms to wheat is

ω-5 gliadin (Tri a 19) (4, 50, 124). Among the timothy allergic children only two had IgE-ab to Tri a 19. One of them had a rather high level (4.1kU$_A$/l) but both children tolerated wheat.

5.3.2 Inhibition test

In paper lll we used timothy pollen extract to inhibit the IgE-ab response to wheat. We could demonstrate complete inhibition in only a minority of patients, whilst inhibition of the remaining sera varied a lot, indicating partial cross-reactivity between the two allergens. Profilin and CCD are two known cross-reactive allergens present in both wheat and timothy pollen and cross-reactive responses due to these allergens have been suggested but not proven (9, 125, 126). In our study about a third of the children displaying sensitization to both wheat and timothy had IgE-ab to profilin and/or CCD. This means that the dual sensitization pattern only partially can be explained by cross-reactivity. Cross-reactivity depending on sensitization to profilin and CCD was also to our knowledge for the first time demonstrated in this study. This finding that we could only partial explain the cross-reactivity was somewhat surprising. It is therefore necessary to continue to search for alternative explanation of these unknown cross-reacting allergens.

We conclude regarding cross-reactivity between timothy and wheat that most children sensitized to foods in our study are also co-sensitized to at least one aeroallergen. Another study found similar serological pattern in a large group of children referred from primary care for allergy testing (127). They found that 75% of the children sensitized to wheat were also sensitized to timothy pollen. Of the children sensitized to timothy pollen, 19% were sensitized to wheat. Our corresponding figures were 73% and 60% respectively. The higher numbers reported by us could be explained by that all our subjects had a doctor's diagnosis of wheat or timothy allergy respectively. This might be interpreted that the more pronounced and diagnosed allergic disease you have the higher likelihood of having a co-sensitization for timothy pollen and wheat. It is well known that birch pollen allergy is often associated with food allergy, mainly to nuts, fruit and vegetables due to cross-reactivity with pathogenesis related allergen-10 (PR-10). Almost two thirds of patients in our study had a simultaneous allergy to birch pollen and 87 % were also sensitized to birch.

Current food allergy was reported by 60 % and out of these nearly all reported allergy to peanut and/or hazelnut. The most surprising finding was that more than half of the subjects, apart from the birch pollen related food allergies, also claimed that they were allergic to staple foods such as milk, egg, soy and fish.

Furthermore, the wheat sensitized individuals reported significantly more often both current and past food allergy to staple foods than those who were non-sensitized to wheat. One way to interpret these results is that wheat sensitization may be a marker of current or past IgE-mediated food allergy in pollinosis patients. Our findings that presence of wheat sensitization in timothy pollen allergic patients should not just be regarded as cross-reactions due to pan-allergens

5.4 HRQL IN CHILDREN WITH WHEAT RESPECTIVE TIMOTHY ALLERGY

We have compared health related quality of life (HRQL) in families with children allergic wheat to families with timothy allergic children. The main difference was that the parents with a wheat allergic child scored significantly lower than parents with a timothy allergic child in several of the domains investigated. These items were general behaviour, general health perceptions, parental impact of emotions, and time, as well as, family activities. This could be interpreted as food allergy causes more stress to the family due to the

increased risk for more severe reactions, including anaphylaxis. Also the activities of daily life are potentially impacted by issues such as label reading of commercial food products, concerns for cross-contamination, careful cooking at home and worries about school canteens. The results are supported by the findings by Cummings et al. that having a child with nut allergies had a significant impact on psychological distress and HRQL for the mothers (128) (3).

Our findings for the wheat allergic group are comparable with the findings for an American group of food allergic children (27). Both our Swedish group and the American group had similar scores for General health, Parental impact-Emotional and Family activities. However, our group had lower scores for Parental impact-Time compared to the American group (61 *vs*. 85), *eg.* Swedish families spend more time due to food allergy compared to American families. A possible explanation could be that the Swedish children are younger, median age; 5 years *vs*. 11 years in the American group, and therefore need more attention. Another explanation is that the most common food allergy in the American group was peanut allergy. Both peanut and wheat allergy demand strict avoidance but wheat allergy probably in addition has a larger impact on home cooking as wheat is a staple food.

Another interesting finding is the difference of the General health perception as the American families report lower score (60 *vs.* 82) compared to the Swedish parents. The description of a low score is that the child believes it´s health is poor and is likely to get worse. One reason could be that the American sample may be biased toward a more severe allergic phenotype as the subjects were recruited by the Food Allergy and Anaphylaxis Network.

To the best of our knowledge, Child Health Questionnaire has not been used in children with allergic rhinitis due to timothy pollen allergy although it is commonly used in asthma studies. One study found that especially in children with persistent wheezing the HRQL was affected, particularly in general health perceptions and physical domains (129). Norrby and co-workers have also studied HRQL in Swedish children diagnosed with asthma (115). When comparing the results with our findings, parents in our timothy group report higher score than parents to asthmatic children in Self Esteem and General Health Perception. This is surprising as almost 80% of the timothy allergic children in our study reported asthma as a symptom. This might be explained by a difference in severity of asthma symptoms, i.e. that our group had less severe symptoms.

Generally there was a better correlation between the timothy allergic children's and parents' perception of HRQL than between wheat allergic children´s and their parents', in our study. Higher correlations between parents and children's responses specifically on Physical functioning were found for the timothy group . This was also seen in the asthmatic group described by Norrby et al. Low correlation between children and parents for our timothy group and for the asthma group in Norrby's study were found for the General behaviour, Role functioning-Emotional and Role functioning-Behaviour domains. This might indicate that it is more difficult for parents to evaluate their children's psychological health than the physical health. Children and parents do not necessarily share similar views about the impact of illness. It is important to directly involve children and teenagers in the management and care, when we meet them as patients at our allergy clinics.

5.5 STRENGTHS AND WEAKNESSES OF THE PRESENT INVESTIGATIONS

5.5.1 Study design

The results should be interpreted with caution since they are based on a limited number of patients but we believe that it raises interesting questions that need long-term follow-up.

A strength of our study is that all patients in the wheat group had a doctors diagnose of wheat allergy hence they all were on a diet without wheat. All patients were also sensitized to wheat. An oral food challenge was performed in all patients except six who had a convincing history of anaphylactic reactions during the last year. A weakness is that these groups of patients are selected and not population based.

In the timothy group all patients had a doctors diagnose of timothy allergy, they were sensitized to timothy and they all tolerated wheat. Again these patients represent a selected group and are not population based. One limitation of this study is that a majority of the subjects were identified and recruited from pediatric university clinics and less so from pediatric primary care clinics. A consequence of this might be that our subjects are more likely to have more severe timothy pollen allergy and associated co-morbidities.

5.5.2 OFC

In research the DBPCFC is regarded as a golden standard for diagnosis of food/wheat allergy. However the procedure is laborious and requires skilled personnel and comes with a risk for the patient. A limitation of this study (paper I, II) is that all the wheat challenges were performed as open food challenges but since the Astier scoring method only takes objective symptoms into consideration, we regard this challenge procedure only to be a minor drawback in the study design. Strength is that all challenges were made in the same unit with same experienced personnel and with a trained allergologist present. Furthermore, when performing the oral challenge test, we have used ordinary wheat products which have been heated when produced. Hence, we cannot exclude that the wheat proteins have undergone changes in this process.

5.5.3 CD-sens

The strength is that the wheat challenges and CD-sens analyses have been performed and evaluated by the same experienced personnel.

Non and low-responders in CD-sens have been described previously (113, 130, 131) and is a weakness of the method. In our study one patient with a positive challenge test was a non-responder in CD-sens and in the group of patients negative in the challenge test one was a non-responder and one a low-responder. The consequence of this is that an oral challenge has to be performed on individuals who are low- or non-responders in the CD-sens test.

We used the recombinant form of ω-5 gliadin, for stimulation of basophils, but had some solubility problems. The basophils require a physiological pH in contrast to ω-5 gliadin, which should be dissolved in 1% acetic acid. Since we may have encountered solubility problems, we could not guarantee the exact amount of allergen in the solutions used and we therefore chose to describe CD-sens to ω-5 gliadin only as positive or negative.

5.5.4 Blood samples and component analysis

The time between challenge and blood sampling should not have exceeded 6 months due to the increased possibility that the child's target organ sensitivity might have changed, i.e. developed tolerance. Three children exceeded this time interval, but they were not excluded because they all had a negative challenge, which had no consequence for the results. The IgE-ab concentration for wheat might not have been affected because most children had been avoiding wheat for a long time. IgE-ab concentration for timothy might have been affected due to the pollen seasonality.

5.5.5 HRQL

The major strength of this study is the validated questionnaires CHQ, which has been used in several studies. A generic instrument is useful for determining the relative burden of a disease and made it possible for us to compare two forms of allergies. Disease-specific tools are useful due to their increased sensitivity of disease-specific issues. We did not use a disease specific questionnaire as our purpose was to compare the atopic conditions.

We adjusted for factors that might impact the results, such as age, gender, and asthma and pollen season. However, it was not possible to adjust for education and economy, which might influence the HRQL (132-134) which is a limitation of the study. Another limitation is the comorbidity within our two study groups in regards to allergic diseases. The wheat allergic individuals had pollen allergies and the pollen allergic individuals had other food allergies except for wheat allergy. This is a real life study and it would be difficult to find subjects with only one condition. This is a real life study and it would be difficult to find subjects with only one condition. A third limitation is the age distribution in the two groups, with younger children in the wheat group in comparison with the timothy group. We have therefore interpreted the results from the wheat allergic children with caution.

6 CONCLUSION

Based on the studies presented, the following conclusions can be drawn:

- Only half of the children challenged with wheat were considered having a wheat allergy and consequently many children were unnecessarily on a wheat-free diet.
- In our population, the level of IgE-ab to ω-5 gliadin, gliadin, HMW-glutenin, and/or LMW-glutenin discriminate wheat allergic from only wheat sensitized children and also seem to predict the severity of the wheat allergy.
- Basophil stimulation in vitro (CD-sens) in combination with IgE-ab to wheat components may be useful when diagnosing wheat allergy.
- A majority of timothy pollen allergic children had low levels of IgE-ab to wheat but tolerated wheat. This dual sensitization is thought to be due to cross-reactivity between timothy pollen and wheat but could only be confirmed in a minority of children in inhibition studies.
- Wheat allergy most likely affect health-related quality of life (HRQL) more than timothy allergy, based on parent's perception.
- Parent and child perceptions of HRQL appear to be more inconsistent among families with wheat allergic, compared to timothy allergic children.

7 CLINICAL UTILITY

In our study only 50 % of the children diagnosed with wheat allergy turned out to have a wheat allergy. This reinforces the need for identifying true wheat allergic children thus being able to remove the food restrictions for those who are not wheat allergic. We have shown a good correlation between an OFC and IgE-ab to wheat-specific proteins in determining true wheat allergy patients.

Quantification of IgE-ab to ω-5 gliadin, gliadin, HMW-glutenin and LMW-glutenin all seem clinically useful in our study population. By combining results from the ω-5 gliadin test, having the highest specificity, with the results for gliadin and HMW-glutenin tests, having the highest sensitivities, it was possible to identify all children in the positive challenge group. Unfortunately some components we have used are not commercially available which means that OFC in clinical practice remains the best option to diagnose true wheat allergy.

Our HRQL investigations clearly showed that families with a wheat allergic child judged their HRQL significantly worse than families with a timothy allergic child. This emphasizes the importance of a correct diagnosis in order to minimize the number of patients with food restrictions.

8 FUTURE PERSPECTIVES

Findings of this thesis show that wheat allergy is over-diagnosed among children and has consequences as impaired quality of life in general for the families. What we do not know yet is how specific food-related situations affect the quality of life in families with a wheat allergic child. Disease-specific questionnaires have been used for other food allergens such as milk, egg and peanut but not in a wheat allergy study. Our intention is to document also these aspects in order to distinguish between food allergic impact and impact from comorbidity.

We found that half of the WA group reacted with severe symptoms and they are at risk of anaphylaxis if exposed accidently to wheat protein. This is a group that potentially would benefit from oral immunotherapy and some attempts have been made globally to treat this patient group. Using omalizumab as protection during wheat oral immunotherapy would be an option in order to avoid severe adverse events.

A new and intriguing problem is the unintentionally ingestion of hydrolysed wheat by the wheat allergic patients. We can show that the basophils in WA patients react to HWP as well as to wheat. That is an indication that HWP would eventually harm the WA patients if exposed. What needs to be done is to document if occasions occur in this group of patients and the severity of the reactions.

Serology for wheat components is an expanding immunological field and we have documented the clinical utility of some gluten-derived components. What further needs to be documented is the role of IgE-ab measurements to these gliadin and glutenin components in the cross-reactivity reactions between wheat and the other grains such as rye, barley and corn. We know that the prolamins are present in those grains. Can the IgE-ab measurement for these allergens be useful in helping the clinician to advise the WA patients what other grain should be avoided or can be included in the diet?

We could not fully explain the cross reactivity between wheat and timothy pollen and that is an area that needs to be exploited in the future. This might not be clinically relevant in terms of management of the WA patient but is important in the improvement of diagnosis of wheat and timothy allergies in order to avoid over--diagnosis as of today. We found that some wheat allergic patients were CD-sens positive to timothy even though they had no symptoms of pollen allergies. It would be of interest to follow up these patients in order to evaluate if this method could predict a future pollen allergy.

9 SVENSK SAMMANFATTNING

Bakgrund:
Överkänslighet mot baslivsmedel som vete, är vanligt förkommande hos små barn. Veteallergi är en svår diagnos att ställa. Att en patient är sensibiliserad (har IgE antikroppar (IgE-ak) i blodet) mot vete utgör en osäker grund för om patientens behov av en vetefri kost eller ej. I praktiken tolkas ofta sensibilisering som allergi och patienterna får strikta dietrestriktioner vilket är näringsmässigt ogynnsamt och innebär en försämring av livskvaliteten. Allergin kan diagnosticeras med hjälp av patientens sjukhistoria, blodprov och/eller hudprick test samt via födoämnesprovokation. Ett positivt blodprov (IgE-ak) eller hudprick test, mot vete, har dock ett begränsat värde för att avgöra om patienten riskerar att få allergiska symtom vid intag av vete. Individer sensibiliserade mot vete kan uppvisa vete IgE-ak pga. gräs som tillhör samma växtfamilj som vete (Poaceae). Veteproteinet ω-5 gliadin är välkänt protein. De individer som har IgE-ak mot detta protein anses vara allergiska mot vete och kan reagera med allvarlig reaktion. Endast var tredje veteallergiker har IgE-ak mot ω-5 gliadin. Vi behöver därför identifiera IgE-ak mot andra veteproteiner.

Basofil stimulering i provrör med allergen mäter basofilcellernas känslighet och är en annan diagnostisk metod som kallas CD-sens och har visat sig vara användbar vid diagnostik av t ex jordnötsallergi. Med denna metod kan man bestämma den minsta mängden av allergenet som stimulerar basofila celler.

Syfte:
Att karaktärisera barn och ungdomar med en läkardiagnostiserad veteallergi immunologiskt och kliniskt samt att studera hur diagnostiken kan förbättras. Att undersöka korsreaktiviteten mellan vete och gräs genom att analysera IgE-ak hos barn och ungdomar med gräspollenallergi.
Att kartlägga dessa två patientgruppers livskvalité.

Material och metoder:
63 barn med diagnosen veteallergi (vetegruppen) och 72 gräsallergiska barn (gräsgruppen) svarade på en livskvalitetsenkät och lämnade blod. Vetegruppen genomgick en öppen veteprovokation, patienterna fick under kontrollerade former, äta stigande mängder av vete. IgE-ak mot ω-5 gliadin, lågmolekylärt glutenin (LMW-glutenin), högmolekylärt (HMW-glutenin) och mot α-, β-, γ-, and ω-5 gliadin (gliadin) analyserades i båda grupperna. Blodprov för CD-sens togs för 24 individer i vetegruppen. Inhibition används för att analysera om det föreligger en korsreaktivitet mellan vete och gräs. Detta görs genom att man till patientens serum tillsätter ett extrakt av ett allergen som binder antikroppar mot etta allergen och sedan mäter man den återstående aktiviteten.

Resultat:
Hälften av de veteallergiska individerna tålde vete vid provokationen (non-WA). IgE-ak nivån mot ω-5 gliadin hos de som uppvisade allergiska symtom vid provokationen (WA) var signifikant högre jämfört med non-WA. Alla barn i WA gruppen hade IgE-ak mot ω-5 gliadin, låg resp. högmolekylärt glutenin och/eller gliadin. Vi kunde även se en positiv korrelation mellan nivån av IgE-ak mot dessa komponenter och allvarlighetsgraden av reaktionen. Flertalet gräsallergiker hade låga nivåer av IgE-ak mot vete (median 0.52 kU_A/l) och 87 % hade IgE-ak mot björk. Genom inhibition undersökte vi om korsreaktivititen mellan gräspollen och vete kunde förklaras av två allergen vanligt förekommande i gräs profillin och CCD. Dessa kunde förklara korsreaktiviteten endast i en tredjedel av fallen. Både de med vete- och gräsallergi var positiva i CD-sens med stimulering med vete respektive gräs. Det fanns en tendens till högre CD-sens värden mot vete hos WA gruppen

jämfört med non-WA. Vetegruppen hade generellt sämre livskvalité än gräsgruppen. Föräldrarna i vetegruppen upplevde en signifikant sämre livskvalitet jämfört med gräsgruppen. Barn och föräldrar i gräsgruppen hade en god överenstämmelse i sin rapportering men den var sämre i vetegruppen.

Slutsats:

Utifrån resultatet i denna avhandling verkar hälften av individerna med en läkarkonstaterad veteallergi undvika vete i onödan. Anledningen till detta kan vara att de utvecklat tolerans med tiden eller att individen felaktigt diagnosticerats pga. korsreaktivitet med gräspollen. Vi har visat att analysera IgE-ak mot gliadiner och gluteniner ökar den diagnostiska träffsäkerheten för veteallergi och skiljer ut de med veteallergi från de med IgE-ak mot vete pga. korsreaktivitet med gräs. CD-sens med vete har ett begränsat användningsområde men kanske kan ge mer om enskilda veteproteiner används för stimulering. Livskvalitén var försämrad i familjerna med ett veteallergiskt barn jämfört med familjerna med en gräsallergisk individ. Detta förstärker ytterligare det faktum att diagnostiken av veteallergi behöver förbättras för att undvika eliminationskost och oro kring födoämnesallergi.

10 ACKNOWLEDGEMENTS

First and foremost I want to thank all children, and their parents, who participated in my studies. Without you it would not been possible to accomplish the increased understanding of wheat allergy.

Magnus P Borres, my main supervisor, thanks for all your support during the years I have devoted to research. Your ideas and positive approach to find solutions has been an inspiration. Thanks to you I have been able to build a network within my research area both within Sweden as well as internationally.

Gunilla Hedlin, my colleague and mentor, you have always been there for me from the day you hired me and hopefully also in the future. Thanks to your belief in me, and for you, I have had the ambition to perform both in the clinic at ALB and in research. I can't even begin to mention all what you have done for me.

Caroline Nilsson, your down to earth support in the practical part of my research as well as your huge input in writing my papers have been incredibly valuable.

Eli Gunnarson, my mentor in research. Thanks for all your wise thoughts and opinions on research and for the interesting and optimistic discussion about life itself. And for your great support in the final stage of the work with my thesis.

Sigrid Sjölander, my co-author, thanks for all your support that made sure that articles were submitted in good order, all strategic discussions and, not least, all statistical calculations. It was really inspiring days we spent together discussing my research in Uppsala.

Anna Nopp, another of my co-authors, thanks to your introduction I have learnt a lot about the world of immunology, basophils and other "immunoreactive" cells and , of course, CD-sens. A special thanks for your practical support in the CD-sens study and in writing the article.

Malin Berthold, nothing escapes your eyes. Highly appreciated input, wise thoughts and reflections. We missed your presence when you were not present.

Gunnar Lilja, it was your idea that we needed to deepen our understanding of wheat allergy. Now we have accomplished part of that and my studies are now formally finished.

Helena Ekoff, you introduced me to inhibition methods, now I have experience of holding pipettes in my hands. Also, thanks for your unbelievable work with the analyses and your patience!!

Yes, research is a part of my universe but equally important is my work at ALB.
Good or not, but the hospital has been my second home and I appreciate all my colleagues and friends I have been working with from different departments and specialities. I can't mention you all but a few:

Anders Lindfors, my role model as paediatrician who "almost always" have had time for good advices and support. One can always learn from you.

Sten Erik Bergström, my absolute favourite paediatric lung specialist!! Knows how to think things through and get them together. Your positivity and your good humour make life easier!

Maria Ingemansson, my colleague who always have answers and good advices. Your patience and accuracy will ensure that your research will be perfect! Good luck!

Eva Horemuzova, my roommate since many years with whom I have had so many nice discussions which always make me feel positive. Thanks for the mental support.

My Finnish colleagues, **Mika Mäkelä, Kati Palosuo** – Thanks for your warm welcome and the inspiring days I spent with you.

Rebecka Lagercrantz, thanks for the positive energy that you share and also for your wonderful paintings that warm my heart – not least"happy children with cinnamon buns" illustrating this thesis.

Helena Olsson, my friend and colleague, thanks for all the happy moments we have had both at work as well as outside work, including long brisk walks in Dubai

Anna-Karin Riis and Ann Berglind, my nurses at Lung/Allergy unit, thank you for all your incredible efforts through these years specially with taking care of the patients. Your being there always makes me feel comfortable. I am also grateful for all your logistical work.

Jeanette, Margaret, thanks for your part in taking care of the patients we had in common.

I also want to thank **Björn Nordlund**, **Sophie Michaelsson**, **Helena Feldt** and **Ashraf K. Persia** for your participation and patience during the years that have passed.

Mari Just; thanks for your thoughts and support. I am sure we will have more time going forward.

Lena Hjelte; My manager since many years, thanks for your support both when it comes to research or clinical work. I appreciate that very much.

Johan Karme; it is rewarding to have a leader who is so down to earth. Thanks!

Henrik Ljungberg, John Konradsen, Anna Asarnoj, Wilhelm Zetterquist, Daiva Helander, Peivi Söderman, my colleagues from Solna and Huddinge as well as my young colleagues from Sachsska Barnsjukhuset **Susanne Glaumann** and **Mirja Vetander**, I really have appreciated interacting with you during the allergy meetings.

I also want to thank all **the ST physicians** from ALB that I have had the pleasure of tutoring at ALB during recent years. It has always been a pleasure and you have contributed to my development. You are so talented.

I also want to thank my physiotherapists; **Åsa, Helena, Christina** the counsellor, the dietitians**; Therese** and **Jenny**. Together we constitute fantastic team.

In department Q63; chief nurses **Åsa** and **Anna**- how should I be able to cope without you, your humility and understanding, you are simply incredible.

Of course I also want to thank all **the staff at Q63**, it is an honour to work with you.

My corridor colleagues and in the diabetes clinic-thanks for all the quick chats that we manage to squeeze in.

All of my friends, both in Sweden ***(Astra Shakira, Loreta & Martins Nigals , Anda Rudzite, Laura Gintere-Kastbom, Aija Landegren, Inta Heimdal&Mickael, Baiba & Andis Lapini, Juris, Lena & Kalle, Pernilla Irestedt, Gita Bolt, Linda & Nicklas Nyman and their family)*** *and in Latvia* ***(Baiba Anderssone, Sandra Rube, Liga Kohtanene),*** *you are very important in all the wonderful events we share including cultural activities****,*** *memories or plain crazy things.*

But that is what makes life worth living!!

My best friend **Aija Brameus**, I really miss our moments together, the lengthy discussions about the stumble blocks of life, our travels together with the children, all the crazy things we have experienced I the US as well as on our journeys. I miss your girls, my "nieces". And of course Claes patience. Wherever you are, you are in my thoughts. **Aijas och Claes parents, they are grate**.

Jolanda Geijer, you are a really good and faithful friend. I am sure we will continue our walks and talks.

My childhood friends (**Martins and Johans Urga**), regardless where we are and under all conditions; you are always "personae gratae".

Elisabeth and **Kjell Öberg**, for all the relaxing times we have had together in Spain, and a special thanks to Kjell for the perfect introduction to surströmming.

Kristina and Sven Erik Håkansson, even though we have met less frequent lately, I have always enjoyed our meetings ever since my first day in Sweden. Even if a long time has passed since we last met it feels like we just continue where we left off.

Catarina and Johan Almqvist, thanks for the nice dinners and the discussions both on the west coast and in Stockholm. I really hope we will keep on meeting one another.

My family, my wonderful parents **Dzintra &Talis** , who always found time to be there for me and giving support when it was needed. Who should I have been without your guidance through life, your values, your intelligence and your demands on me?

My sister **Dace** , I always looked up to you as a child and strived to reach your level, which was very encouraging for me. Thanks for all the long walks filled with discussions of everything worth discussing. I feel I can always trust you.

My little brother **Raimonds**, life has not always been easy, but most important is to make the right choices.

My godmother and my aunt for always caring about me and for your warm thoughts.

My relatives from Cesis in Latvia **Liga, Raita, Janis, Ilmars and Sarmite.**

Gunta Putna, my faithful friend and lawyer, for your wisdom, loyalty and diplomacy through all the years we have known each other.

I want to thank Jan's family, his **father, Ulla, Lars, Ingela, Kerstin, Claes** and families –

I have always felt like being part of a big family.

Jan's children, **Fredrik** and his family, **Åsa** and the boys, **Erik** - we have had many enjoyable moments in Mollösund and in London.

Ann, thanks for all the long discussions that has empowered me, giving me positive charge and a belief in myself when needed most.

Jan: my life's best friend. Thanks for your patience, intelligence, support, wisdom and values. Your importance in forming me to what I am today is beyond description. I can always trust you and I am always proud of you. You changed my life.

Johan and Gustav, my beloved children. You are my greatest challenge and I am not sure if I learn more from you or vice versa – probably both. I will always be there for you**. I love you so much! You are the best, I always believe in you!**

Founding sources

This research project had not been possible without financial support from:

Centre for Allergy research at Karolinska Institutet, Hesselman Foundation, Foundation Smariten, Swedish Asthma and Allergy Association`s, Foundation Frimurarna, Princess Lovisa`s Association for Children`s Medical Care and Foundation "Mjölkdroppen".

11 REFERENCES

1. Wang J, Sampson HA. Food allergy. J Clin Invest. 2011;121(3):827-35.

2. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol. 2007;120(3):638-46.

3. Linna O. Specific IgE antibodies to uningested cereals. Allergy. 1996;51(11):849-50.

4. Palosuo K, Varjonen E, Kekki OM, Klemola T, Kalkkinen N, Alenius H, et al. Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. J Allergy Clin Immunol. 2001;108(4):634-8.

5. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol. 2001;107(5):891-6.

6. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. Clin Exp Allergy. 2005;35(3):268-73.

7. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol. 1997;100(4):444-51.

8. Jones SM, Magnolfi CF, Cooke SK, Sampson HA. Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity. J Allergy Clin Immunol. 1995;96(3):341-51.

9. Matricardi PM, Bockelbrink A, Beyer K, Keil T, Niggemann B, Gruber C, et al. Primary versus secondary immunoglobulin E sensitization to soy and wheat in the Multi-Centre Allergy Study cohort. Clin Exp Allergy. 2008;38(3):493-500.

10. Glaumann S, Nopp A, Johansson SG, Borres MP, Nilsson C. Oral peanut challenge identifies an allergy but the peanut allergen threshold sensitivity is not reproducible. PLoS One. 2013;8(1):e53465.

11. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V, et al. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. J Allergy Clin Immunol. 2015;135(1):179-86.

12. van der Velde JL, Flokstra-de Blok BM, de Groot H, Oude-Elberink JN, Kerkhof M, Duiverman EJ, et al. Food allergy-related quality of life after double-blind, placebo-controlled food challenges in adults, adolescents, and children. J Allergy Clin Immunol. 2012;130(5):1136-43 e2.

13. Morou Z, Tatsioni A, Dimoliatis ID, Papadopoulos NG. Health-related quality of life in children with food allergy and their parents: a systematic review of the literature. J Investig Allergol Clin Immunol. 2014;24(6):382-95.

14. King RM, Knibb RC, Hourihane JO. Impact of peanut allergy on quality of life, stress and anxiety in the family. Allergy. 2009;64(3):461-8.

15. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol. 2004;113(5):832-6.

16. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy. 2001;56(9):813-24.

17. Zuidmeer L, Goldhahn K, Rona RJ, Gislason D, Madsen C, Summers C, et al. The prevalence of plant food allergies: a systematic review. J Allergy Clin Immunol. 2008;121(5):1210-8 e4.

18. Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. Allergy. 2014;69(1):62-75.

19. Santos AF, Lack G. Food allergy and anaphylaxis in pediatrics: update 2010-2012. Pediatr Allergy Immunol. 2012;23(8):698-706.

20. Prescott S, Allen KJ. Food allergy: riding the second wave of the allergy epidemic. Pediatr Allergy Immunol. 2011;22(2):155-60.

21. Eigenmann PA, Sicherer SH, Borkowski TA, Cohen BA, Sampson HA. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. Pediatrics. 1998;101(3):E8.

22. Igea JM. The history of the idea of allergy. Allergy. 2013;68(8):966-73.

23. Valenta R, Hochwallner H, Linhart B, Pahr S. Food allergies: the basics. Gastroenterology. 2015;148(6):1120-31 e4.

24. Burks AW, Tang M, Sicherer S, Muraro A, Eigenmann PA, Ebisawa M, et al. ICON: food allergy. J Allergy Clin Immunol. 2012;129(4):906-20.

25. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the Diagnosis and Management of Food Allergy in the United States: Summary of the NIAID-Sponsored Expert Panel Report. J Allergy Clin Immunol. 2010;126(6):1105-18.

26. Ostblom E, Egmar AC, Gardulf A, Lilja G, Wickman M. The impact of food hypersensitivity reported in 9-year-old children by their parents on health-related quality of life. Allergy. 2008;63(2):211-8.

27. Sicherer SH, Noone SA, Munoz-Furlong A. The impact of childhood food allergy on quality of life. Ann Allergy Asthma Immunol. 2001;87(6):461-4.

28. Delves PJ, Roitt IM. The immune system. Second of two parts. N Engl J Med. 2000;343(2):108-17.

29. Chaplin DD. Overview of the immune response. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S3-23.

30. Rindsjo E, Scheynius A. Mechanisms of IgE-mediated allergy. Exp Cell Res. 2010;316(8):1384-9.

31. Galli SJ, Tsai M. IgE and mast cells in allergic disease. Nat Med. 2012;18(5):693-704.

32. Han Y, Kim J, Ahn K. Food allergy. Korean J Pediatr. 2012;55(5):153-8.

33. Ortolani C, Ispano M, Pastorello E, Bigi A, Ansaloni R. The oral allergy syndrome. Ann Allergy. 1988;61(6 Pt 2):47-52.

34. Lack G. Update on risk factors for food allergy. J Allergy Clin Immunol. 2012;129(5):1187-97.

35. Charlesworth EN. Allergic skin disease: atopic dermatitis as a prototype. Prim Care. 1998;25(4):775-90.

36. Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. J Allergy Clin Immunol. 2007;120(5):1172-7.

37. Savage JH, Matsui EC, Skripak JM, Wood RA. The natural history of egg allergy. J Allergy Clin Immunol. 2007;120(6):1413-7.

38. Shek LP, Soderstrom L, Ahlstedt S, Beyer K, Sampson HA. Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy. J Allergy Clin Immunol. 2004;114(2):387-91.

39. Kotaniemi-Syrjanen A, Palosuo K, Jartti T, Kuitunen M, Pelkonen AS, Makela MJ. The prognosis of wheat hypersensitivity in children. Pediatr Allergy Immunol. 2010;21(2 Pt 2):e421-8.

40. Shreffler WG, Wanich N, Moloney M, Nowak-Wegrzyn A, Sampson HA. Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. J Allergy Clin Immunol. 2009;123(1):43-52 e7.

41. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. J Exp Med. 2004;199(12):1679-88.

42. Campbell DE, Boyle RJ, Thornton CA, Prescott SL. Mechanisms of allergic disease - environmental and genetic determinants for the development of allergy. Clin Exp Allergy. 2015;45(5):844-58.

43. Breiteneder H, Ebner C. Molecular and biochemical classification of plant-derived food allergens. J Allergy Clin Immunol. 2000;106(1 Pt 1):27-36.

44. Wieser H. Chemistry of gluten proteins. Food Microbiol. 2007;24(2):115-9.

45. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, et al. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med. 2012;10:13.

46. Brisman J. Baker's asthma. Occup Environ Med. 2002;59(7):498-502; quiz , 426.

47. Keet CA, Matsui EC, Dhillon G, Lenehan P, Paterakis M, Wood RA. The natural history of wheat allergy. Ann Allergy Asthma Immunol. 2009;102(5):410-5.

48. Czaja-Bulsa G, Bulsa M. The natural history of IgE mediated wheat allergy in children with dominant gastrointestinal symptoms. Allergy Asthma Clin Immunol. 2014;10(1):12.

49. Morita E, Matsuo H, Chinuki Y, Takahashi H, Dahlstrom J, Tanaka A. Food-dependent exercise-induced anaphylaxis -importance of omega-5 gliadin and HMW-glutenin

as causative antigens for wheat-dependent exercise-induced anaphylaxis. Allergol Int. 2009;58(4):493-8.

50. Inomata N. Wheat allergy. Curr Opin Allergy Clin Immunol. 2009;9(3):238-43.

51. Varjonen E, Vainio E, Kalimo K. Antigliadin IgE--indicator of wheat allergy in atopic dermatitis. Allergy. 2000;55(4):386-91.

52. Matsuo H, Morimoto K, Akaki T, Kaneko S, Kusatake K, Kuroda T, et al. Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-dependent exercise-induced anaphylaxis. Clin Exp Allergy. 2005;35(4):461-6.

53. Aihara M, Miyazawa M, Osuna H, Tsubaki K, Ikebe T, Aihara Y, et al. Food-dependent exercise-induced anaphylaxis: influence of concurrent aspirin administration on skin testing and provocation. Br J Dermatol. 2002;146(3):466-72.

54. Lauriere M, Pecquet C, Bouchez-Mahiout I, Snegaroff J, Bayrou O, Raison-Peyron N, et al. Hydrolysed wheat proteins present in cosmetics can induce immediate hypersensitivities. Contact Dermatitis. 2006;54(5):283-9.

55. Sanchez-Perez J, Sanz T, Garcia-Diez A. Allergic contact dermatitis from hydrolyzed wheat protein in cosmetic cream. Contact Dermatitis. 2000;42(6):360.

56. Olaiwan A, Pecquet C, Mathelier-Fusade P, Frances C. [Contact urticaria induced by hydrolyzed wheat proteins in cosmetics]. Ann Dermatol Venereol. 2010;137(4):281-4.

57. Varjonen E, Petman L, Makinen-Kiljunen S. Immediate contact allergy from hydrolyzed wheat in a cosmetic cream. Allergy. 2000;55(3):294-6.

58. Fukutomi Y, Itagaki Y, Taniguchi M, Saito A, Yasueda H, Nakazawa T, et al. Rhinoconjunctival sensitization to hydrolyzed wheat protein in facial soap can induce wheat-dependent exercise-induced anaphylaxis. J Allergy Clin Immunol. 2011;127(2):531-3 e1-3.

59. Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A, et al. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. Allergy. 2014;69(8):992-1007.

60. Venter C, Pereira B, Voigt K, Grundy J, Clayton CB, Higgins B, et al. Prevalence and cumulative incidence of food hypersensitivity in the first 3 years of life. Allergy. 2008;63(3):354-9.

61. Ostblom E, Lilja G, Ahlstedt S, van Hage M, Wickman M. Patterns of quantitative food-specific IgE-antibodies and reported food hypersensitivity in 4-year-old children. Allergy. 2008;63(4):418-24.

62. Ostblom E, Wickman M, van Hage M, Lilja G. Reported symptoms of food hypersensitivity and sensitization to common foods in 4-year-old children. Acta Paediatr. 2008;97(1):85-90.

63. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008-25.

64. Knight AK, Shreffler WG, Sampson HA, Sicherer SH, Noone S, Mofidi S, et al. Skin prick test to egg white provides additional diagnostic utility to serum egg white-specific IgE antibody concentration in children. J Allergy Clin Immunol. 2006;117(4):842-7.

65. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. Clin Exp Allergy. 2000;30(11):1540-6.

66. Sicherer SH, Sampson HA. 9. Food allergy. J Allergy Clin Immunol. 2006;117(2 Suppl Mini-Primer):S470-5.

67. Roberts G, Lack G. Diagnosing peanut allergy with skin prick and specific IgE testing. J Allergy Clin Immunol. 2005;115(6):1291-6.

68. Sicherer SH, Wood RA, American Academy of Pediatrics Section On A, Immunology. Allergy testing in childhood: using allergen-specific IgE tests. Pediatrics. 2012;129(1):193-7.

69. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, et al. The skin prick test - European standards. Clin Transl Allergy. 2013;3(1):3.

70. Soares-Weiser K, Takwoingi Y, Panesar SS, Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. The diagnosis of food allergy: a systematic review and meta-analysis. Allergy. 2014;69(1):76-86.

71. Ballmer-Weber BK. Value of allergy tests for the diagnosis of food allergy. Dig Dis. 2014;32(1-2):84-8.

72. Norrman G, Falth-Magnusson K. Adverse reactions to skin prick testing in children - prevalence and possible risk factors. Pediatr Allergy Immunol. 2009;20(3):273-8.

73. Cox L. Overview of serological-specific IgE antibody testing in children. Curr Allergy Asthma Rep. 2011;11(6):447-53.

74. Canonica GW, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, et al. A WAO - ARIA - GA(2)LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ J. 2013;6(1):17.

75. Borres MP, Ebisawa M, Eigenmann PA. Use of allergen components begins a new era in pediatric allergology. Pediatr Allergy Immunol. 2011;22(5):454-61.

76. Tatham AS, Shewry PR. Allergens to wheat and related cereals. Clin Exp Allergy. 2008;38(11):1712-26.

77. Simonato B, De Lazzari F, Pasini G, Polato F, Giannattasio M, Gemignani C, et al. IgE binding to soluble and insoluble wheat flour proteins in atopic and non-atopic patients suffering from gastrointestinal symptoms after wheat ingestion. Clin Exp Allergy. 2001;31(11):1771-8.

78. Pastorello EA, Farioli L, Conti A, Pravettoni V, Bonomi S, Iametti S, et al. Wheat IgE-mediated food allergy in European patients: alpha-amylase inhibitors, lipid transfer proteins and low-molecular-weight glutenins. Allergenic molecules recognized by double-blind, placebo-controlled food challenge. Int Arch Allergy Immunol. 2007;144(1):10-22.

79. Makela MJ, Eriksson C, Kotaniemi-Syrjanen A, Palosuo K, Marsh J, Borres M, et al. Wheat allergy in children - new tools for diagnostics. Clin Exp Allergy. 2014;44(11):1420-30.

80. Mamone G, Picariello G, Addeo F, Ferranti P. Proteomic analysis in allergy and intolerance to wheat products. Expert Rev Proteomics. 2011;8(1):95-115.

81. Ebisawa M, Shibata R, Sato S, Borres MP, Ito K. Clinical utility of IgE antibodies to omega-5 gliadin in the diagnosis of wheat allergy: a pediatric multicenter challenge study. Int Arch Allergy Immunol. 2012;158(1):71-6.

82. Nilsson N, Sjolander S, Baar A, Berthold M, Pahr S, Vrtala S, et al. Wheat allergy in children evaluated with challenge and IgE antibodies to wheat components. Pediatr Allergy Immunol. 2015;26(2):119-25.

83. Hoffmann-Sommergruber K, Pfeifer S, Bublin M. Applications of Molecular Diagnostic Testing in Food Allergy. Curr Allergy Asthma Rep. 2015;15(9):56.

84. Baar A, Pahr S, Constantin C, Giavi S, Manoussaki A, Papadopoulos NG, et al. Specific IgE reactivity to Tri a 36 in children with wheat food allergy. J Allergy Clin Immunol. 2014;133(2):585-7.

85. Takahashi H, Matsuo H, Chinuki Y, Kohno K, Tanaka A, Maruyama N, et al. Recombinant high molecular weight-glutenin subunit-specific IgE detection is useful in identifying wheat-dependent exercise-induced anaphylaxis complementary to recombinant omega-5 gliadin-specific IgE test. Clin Exp Allergy. 2012;42(8):1293-8.

86. Sato S, Tachimoto H, Shukuya A, Kurosaka N, Yanagida N, Utsunomiya T, et al. Basophil activation marker CD203c is useful in the diagnosis of hen's egg and cow's milk allergies in children. Int Arch Allergy Immunol. 2010;152 Suppl 1:54-61.

87. Ebo DG, Bridts CH, Hagendorens MM, de Clerck LS, Stevens WJ. The basophil activation test in the diagnosis and follow-up of hymenoptera venom allergy: an alternative point of view. J Investig Allergol Clin Immunol. 2008;18(6):493-4.

88. Sato S, Tachimoto H, Shukuya A, Ogata M, Komata T, Imai T, et al. Utility of the peripheral blood basophil histamine release test in the diagnosis of hen's egg, cow's milk, and wheat allergy in children. Int Arch Allergy Immunol. 2011;155 Suppl 1:96-103.

89. MacGlashan DW, Jr. Basophil activation testing. J Allergy Clin Immunol. 2013;132(4):777-87.

90. Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. J Allergy Clin Immunol. 1991;88(3 Pt 1):328-38.

91. Nopp A, Cardell LO, Johansson SG. CD-sens can be a reliable and easy-to-use complement in the diagnosis of allergic rhinitis. Int Arch Allergy Immunol. 2013;161(1):87-90.

92. Johansson SG, Nopp A, van Hage M, Olofsson N, Lundahl J, Wehlin L, et al. Passive IgE-sensitization by blood transfusion. Allergy. 2005;60(9):1192-9.

93. Pignatti P, Yacoub MR, Testoni C, Pala G, Corsetti M, Colombo G, et al. Evaluation of basophil activation test in suspected food hypersensitivity. Cytometry B Clin Cytom. 2015.

94. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods--position paper from the European Academy of Allergology and Clinical Immunology. Allergy. 2004;59(7):690-7.

95. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and

Clinical Immunology PRACTALL consensus report. J Allergy Clin Immunol. 2012;130(6):1260-74.

96. Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol. 1988;82(6):986-97.

97. Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS, et al. Work Group report: oral food challenge testing. J Allergy Clin Immunol. 2009;123(6 Suppl):S365-83.

98. Cianferoni A, Khullar K, Saltzman R, Fiedler J, Garrett JP, Naimi DR, et al. Oral food challenge to wheat: a near-fatal anaphylaxis and review of 93 food challenges in children. World Allergy Organ J. 2013;6(1):14.

99. Kiotseridis H, Cilio CM, Bjermer L, Aurivillius M, Jacobsson H, Dahl A, et al. Quality of life in children and adolescents with respiratory allergy, assessed with a generic and disease-specific instrument. Clin Respir J. 2013;7(2):168-75.

100. Kiotseridis H, Bjermer L, Pilman E, Stallberg B, Romberg K, Tunsater A. ALMA, a new tool for the management of asthma patients in clinical practice: development, validation and initial clinical findings. Prim Care Respir J. 2012;21(2):139-44.

101. Tripodi S, Frediani T, Lucarelli S, Macri F, Pingitore G, Di Rienzo Businco A, et al. Molecular profiles of IgE to Phleum pratense in children with grass pollen allergy: implications for specific immunotherapy. J Allergy Clin Immunol. 2012;129(3):834-9 e8.

102. Mari A. Skin test with a timothy grass (Phleum pratense) pollen extract vs. IgE to a timothy extract vs. IgE to rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, and rPhl p 12: epidemiological and diagnostic data. Clin Exp Allergy. 2003;33(1):43-51.

103. Constantin C, Quirce S, Poorafshar M, Touraev A, Niggemann B, Mari A, et al. Micro-arrayed wheat seed and grass pollen allergens for component-resolved diagnosis. Allergy. 2009;64(7):1030-7.

104. Patelis A, Gunnbjornsdottir M, Borres MP, Burney P, Gislason T, Toren K, et al. Natural history of perceived food hypersensitivity and IgE sensitisation to food allergens in a cohort of adults. PLoS One. 2014;9(1):e85333.

105. DunnGalvin A, Dubois AE, Flokstra-de Blok BM, Hourihane JO. The effects of food allergy on quality of life. Chem Immunol Allergy. 2015;101:235-52.

106. Wassenberg J, Cochard MM, Dunngalvin A, Ballabeni P, Flokstra-de Blok BM, Newman CJ, et al. Parent perceived quality of life is age-dependent in children with food allergy. Pediatr Allergy Immunol. 2012;23(5):412-9.

107. Mikkelsen A, Borres MP, Bjorkelund C, Lissner L, Oxelmark L. The food hypersensitivity family impact (FLIP) questionnaire - development and first results. Pediatr Allergy Immunol. 2013;24(6):574-81.

108. Protudjer J, Jansson SA, Ostblom E, Arnlind MH, Bengtsson U, Dahlen SE, et al. Health-related quality of life in children with objectively diagnosed staple food allergy assessed with a disease-specific questionnaire. Acta Paediatr. 2015;104(10):1047-54.

109. Salvilla SA, Dubois AE, Flokstra-de Blok BM, Panesar SS, Worth A, Patel S, et al. Disease-specific health-related quality of life instruments for IgE-mediated food allergy. Allergy. 2014;69(7):834-44.

110. Astier C, Morisset M, Roitel O, Codreanu F, Jacquenet S, Franck P, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. J Allergy Clin Immunol. 2006;118(1):250-6.

111. Baar A, Pahr S, Constantin C, Giavi S, Papadopoulos NG, Pelkonen AS, et al. The high molecular weight glutenin subunit Bx7 allergen from wheat contains repetitive IgE epitopes. Allergy. 2014;69(10):1316-23.

112. Nopp A, Johansson SG, Ankerst J, Bylin G, Cardell LO, Gronneberg R, et al. Basophil allergen threshold sensitivity: a useful approach to anti-IgE treatment efficacy evaluation. Allergy. 2006;61(3):298-302.

113. Glaumann S, Nopp A, Johansson SG, Rudengren M, Borres MP, Nilsson C. Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children. Allergy. 2012;67(2):242-7.

114. Raat H, Botterweck AM, Landgraf JM, Hoogeveen WC, Essink-Bot ML. Reliability and validity of the short form of the child health questionnaire for parents (CHQ-PF28) in large random school based and general population samples. J Epidemiol Community Health. 2005;59(1):75-82.

115. Norrby U, Nordholm L, Andersson-Gare B, Fasth A. Health-related quality of life in children diagnosed with asthma, diabetes, juvenile chronic arthritis or short stature. Acta Paediatr. 2006;95(4):450-6.

116. Winberg A, West CE, Strinnholm A, Nordstrom L, Hedman L, Ronmark E. Assessment of Allergy to Milk, Egg, Cod, and Wheat in Swedish Schoolchildren: A Population Based Cohort Study. PLoS One. 2015;10(7):e0131804.

117. Leduc V, Moneret-Vautrin DA, Guerin L, Morisset M, Kanny G. Anaphylaxis to wheat isolates: immunochemical study of a case proved by means of double-blind, placebo-controlled food challenge. J Allergy Clin Immunol. 2003;111(4):897-9.

118. Chinuki Y, Morita E. Wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat protein in soap. Allergol Int. 2012;61(4):529-37.

119. Tokuda R, Nagao M, Hiraguchi Y, Hosoki K, Matsuda T, Kouno K, et al. Antigen-induced expression of CD203c on basophils predicts IgE-mediated wheat allergy. Allergol Int. 2009;58(2):193-9.

120. Wickman M, Asarnoj A, Tillander H, Andersson N, Bergstrom A, Kull I, et al. Childhood-to-adolescence evolution of IgE antibodies to pollens and plant foods in the BAMSE cohort. J Allergy Clin Immunol. 2014;133(2):580-2.

121. Tordesillas L, Pacios LF, Palacin A, Quirce S, Armentia A, Barber D, et al. Molecular basis of allergen cross-reactivity: non-specific lipid transfer proteins from wheat flour and peach fruit as models. Mol Immunol. 2009;47(2-3):534-40.

122. Guillen D, Barranco P, Palacin A, Quirce S. Occupational Rhinoconjunctivitis due to Maize in a Snack Processor: A Cross-Reactivity Study Between Lipid Transfer Proteins From Different Cereals and Peach. Allergy Asthma Immunol Res. 2014;6(5):470-3.

123. Palacin A, Quirce S, Armentia A, Fernandez-Nieto M, Pacios LF, Asensio T, et al. Wheat lipid transfer protein is a major allergen associated with baker's asthma. J Allergy Clin Immunol. 2007;120(5):1132-8.

124. Palosuo K. Update on wheat hypersensitivity. Curr Opin Allergy Clin Immunol. 2003;3(3):205-9.

125. Sander I, Rihs HP, Doekes G, Quirce S, Krop E, Rozynek P, et al. Component-resolved diagnosis of baker's allergy based on specific IgE to recombinant wheat flour proteins. J Allergy Clin Immunol. 2015;135(6):1529-37.

126. Sander I, Rozynek P, Rihs HP, van Kampen V, Chew FT, Lee WS, et al. Multiple wheat flour allergens and cross-reactive carbohydrate determinants bind IgE in baker's asthma. Allergy. 2011;66(9):1208-15.

127. de Jong AB, Dikkeschei LD, Brand PL. Sensitization patterns to food and inhalant allergens in childhood: a comparison of non-sensitized, monosensitized, and polysensitized children. Pediatr Allergy Immunol. 2011;22(2):166-71.

128. Cummings AJ, Knibb RC, Erlewyn-Lajeunesse M, King RM, Roberts G, Lucas JS. Management of nut allergy influences quality of life and anxiety in children and their mothers. Pediatr Allergy Immunol. 2010;21(4 Pt 1):586-94.

129. Hafkamp-de Groen E, Mohangoo AD, Landgraf JM, de Jongste JC, Duijts L, Moll HA, et al. The impact of preschool wheezing patterns on health-related quality of life at age 4 years. Eur Respir J. 2013;41(4):952-9.

130. Ocmant A, Mulier S, Hanssens L, Goldman M, Casimir G, Mascart F, et al. Basophil activation tests for the diagnosis of food allergy in children. Clin Exp Allergy. 2009;39(8):1234-45.

131. Rubio A, Vivinus-Nebot M, Bourrier T, Saggio B, Albertini M, Bernard A. Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children. Allergy. 2011;66(1):92-100.

132. Venter C, Sommer I, Moonesinghe H, Grundy J, Glasbey G, Patil V, et al. Health-related quality of life in children with perceived and diagnosed food hypersensitivity. Pediatr Allergy Immunol. 2015;26(2):126-32.

133. Warren CM, Gupta RS, Sohn MW, Oh EH, Lal N, Garfield CF, et al. Differences in empowerment and quality of life among parents of children with food allergy. Ann Allergy Asthma Immunol. 2015;114(2):117-25.

134. Houben-van Herten M, Bai G, Hafkamp E, Landgraf JM, Raat H. Determinants of health-related quality of life in school-aged children: a general population study in the Netherlands. PLoS One. 2015;10(5):e0125083.