

## SUMMARY

IgE-mediated allergy to wheat proteins can be caused by exposure through ingestion, inhalation, or skin/mucosal contact, and can affect various populations and age groups. Respiratory allergy to wheat proteins is commonly observed in adult patients occupationally exposed to flour, whereas wheat food allergy is more common in children. Wheat allergy is of growing importance for patients with recurrent anaphylaxis, especially when exercise related. The diagnosis of wheat allergy relies on a consistent clinical history, skin prick testing with well-characterized extracts and specific IgE tests. The accuracy of wheat allergy diagnosis may be improved by measuring IgE responses to several wheat components. However, a high degree of heterogeneity has been found in the recognition pattern of allergens among patient groups with different clinical profiles, as well as within each group. Thus, oral provocation with wheat or the implicated cereal is the reference test for the definitive diagnosis of ingested wheat/cereal allergy.

**KEYWORDS:** Cereal allergy, baker's asthma, wheat allergens, food allergy, anaphylaxis.

## INTRODUCTION

Cereals are an important source of dietary protein for individuals worldwide. The most widely used cereal for human consumption is wheat, although other cereals such as rice, corn, barley, rye, oat and, to a lesser extent, sorghum and millet are also consumed. All cereals belong to the *Poaceae* family or *Gramineae*, i.e., they are cultivated grasses and produce edible fruits known as grains. There are numerous and varied foods that contain cereals. In addition to bakery products, pastry and pasta, grains are widely used in the food industry as thickeners and fillers.

The prevalence of cereal allergy has been reported to be between 0.3% and 0.5% among children aged 0 to 14 years [1,2]. Other studies point out that IgE-mediated allergy to wheat affects at least 0.5% of the pediatric population and 1% of the adult population [3].

The diagnosis of hypersensitivity to wheat, and to cereals in general, is complicated by the various pathogenic mechanisms that can be involved. In clinical practice it is important to differentiate whether an adverse reaction to cereal grains is due to an IgE-mediated or non-IgE-mediated allergic reaction, or to a nonallergic hypersensitivity reaction (intolerance).

In this review paper we focus mainly on IgE-mediated allergy to wheat, and to a lesser extent to other cereals. Celiac disease [4] and, more recently, nonceliac gluten sensitivity [5] have been widely addressed elsewhere.

## CLINICAL PRESENTATION

### Clinical signs and symptoms

IgE-mediated allergic reactions to cereal proteins can be caused by exposure through different routes (inhalation, ingestion and/or skin or mucosal contact) and can affect various populations and age groups. Although wheat (*Triticus aestivum*) is the most commonly involved grain in cereal allergy in Western populations, other cereals (rice, corn, rye, barley and oat) can also be implicated.

Respiratory allergy to wheat proteins, usually manifested as rhinitis and baker's asthma, is one of the more common types of occupational asthma [6]. The prevalence of work-related respiratory symptoms is high among bakery workers, about 15-20% suffer rhinitis and 5-10% asthma. Bakers, confectioners, pastry factory workers, pizza makers, millers, farmers, and cereal handlers are the job categories more commonly affected. This condition is mainly due to an IgE-mediated allergic response to inhalation of cereal flour proteins, particularly wheat and rye [6]. Interestingly, baker's asthma patients usually tolerate ingested cereals without any adverse effects, which indicate the importance of the sensitization route in the clinical manifestations. It has been reported that the exposure-response relationships for average exposure follow a linear relationship for sensitisation, but a bell-shaped curve for allergic symptoms and probable occupational asthma [7].

Cereals can be generally introduced in the infant's diet from 4-6 months of life [8], but sensitization can be observed before ingestion due to their passage via breast milk to children who are exclusively breastfed [9]. Patients with typical IgE-mediated food allergy show clinical symptoms that include one or several of the following manifestations: urticaria/angioedema, vomiting, bronchospasm, anaphylaxis and atopic

dermatitis. Wheat allergy manifest frequently in the form of outbreaks of atopic dermatitis in children, and cosensitization to cow's milk and egg is common [10,11]. Wheat allergy has been identified as responsible for the syndrome known as wheat-dependent exercise-induced anaphylaxis (WDEIA) which usually develops after ingestion of wheat products followed by physical exercise or concurrence of other cofactors such as nonsteroidal anti-inflammatory drugs, alcohol, and infections. This condition is clinically characterized by anaphylactic reactions ranging from urticaria and angioedema to dyspnea, hypotension and shock, and occurs when wheat ingestion is accompanied by several cofactors [12]. The main allergens involved in this condition are the gliadins present in the salt-insoluble fraction of wheat flour, specifically omega-5 gliadin, and high-molecular-weight glutenin subunits [13]. Recently, an increased incidence of a new subtype of WDEIA due to sensitization through percutaneous and/or rhinoconjunctival route to hydrolyzed wheat protein (HWP) contained in soap has been described, particularly in Japan [14]. In this syndrome, contact allergy with soap exposure usually preceded wheat ingestion-induced reactions. The most commonly observed symptom of this WDEIA subtype was angioedema of the eyelids, and some patients developed anaphylaxis. These patients have little omega-5 gliadin-specific serum IgE [14]. An epidemiological relationship between wheat allergy and contact exposure to HWP has been documented among Japanese women [15]. This finding implicates a possible role of contact exposure to food-derived protein hydrolysates as a risk factor for the development of food allergy manifesting as anaphylaxis. In addition, food allergy to a wheat isolate has been reported, specifically to deamidated gluten contained in technologically processed cereal products, which may give rise to severe allergic reactions in subjects tolerant to native wheat, and that is characterized by a homogeneous IgE response [16]. Allergy to HWP and food allergy to deamidated gluten can have some similarities regarding the allergens and epitopes involved.

### **Natural history of wheat food allergy**

Cereal allergy is considered one of the types of food allergy that tend to disappear with age, like cow's milk and egg allergy, but until recently there have been few studies confirming this observation.

Kotaniemi-Syrjänen et al [17] analyzed the natural history of wheat allergy in children, to define the risk factors related to the persistence of allergies to this food and to assess the development of respiratory allergies. The development and potential disappearance of wheat allergy, as well as the onset of rhinoconjunctivitis and allergic asthma, were retrospectively assessed in 28 children with wheat allergy demonstrated by an open oral challenge test at a median age of 21 months (range, 6–75 months). Development of cutaneous symptoms during the wheat oral challenge was related to wheat allergy with positive skin prick tests (SPT), whereas the appearance of gastrointestinal symptoms alone (without cutaneous and respiratory symptoms) was associated with negative SPT reactions to wheat. Wheat was tolerated by 59%, 69%, 84% and 96% of the children at 4, 6, 10 and 16 years, respectively. A SPT wheal size to gliadin  $\geq 5$  mm at the time of the oral challenge was associated with a slower course of the remission of wheat allergy, whereas a wheal  $\geq 3$  mm to gliadin at any time was significantly associated with the development of asthma. The authors concluded that almost all children with wheat allergy can tolerate this food in adolescence, and that sensitization to gliadin is associated with a slower development of tolerance and an increased risk of asthma [17].

Keet et al [18] studied the natural course of wheat allergy and sought to identify the factors that help predict the prognosis in a population of 103 children with this allergy. Patients were included in the study if they had a history of symptomatic reactions to this food and a positive wheat-specific IgE test. The rates of resolution, established

according to the results of the challenge tests, were 29% at 4 years, 56% at 8 years and 65% at 12 years. High levels of wheat-specific IgE were associated with a poorer prognosis. The maximum recorded level of wheat-specific IgE was a significant predictor of persistent allergy, although numerous children overcome the wheat allergy even with high wheat-specific IgE levels. In short, the median age of wheat allergy remission was approximately 6.5 years in this population. In a significant minority of patients, the wheat allergy persisted into adolescence.

In contrast, Mansouri et al [19] reported that only 3 of 8 children (37.5%) with wheat food allergy (mostly with anaphylaxis) developed tolerance after 8 years of follow-up, achieving tolerance at a mean age of 5.6 years (range, 3–9 years).

Recently, Czaja-Bulsa et al [20] studied the natural course of wheat allergy in children with dominant gastrointestinal symptoms, as confirmed by food challenge results, and they found that the majority of children with wheat allergy can tolerate wheat by adolescence. The age when tolerance to wheat developed depended on the level and the age of reaching the highest levels of wheat-specific IgE. The higher the values of the above parameters, the older a child was when they developed tolerance to wheat.

## ALLERGENS

Proteins represent approximately 10-15% (dry weight) of the wheat grain and can be classified into 3 different fractions based on the sequential extraction in a series of solvents [21]. The salt-soluble fractions, called albumins and globulins, represent only 15-20% of the total proteins, while the majority of proteins, called prolamins (gliadins and glutenins), are not extractable in saline solutions. Gliadins are monomeric proteins and include 3 types (alpha/beta, gamma and omega), based on their electrophoretic mobility at low pH and their biochemical characteristics [22].

Glutenins form polymers stabilized by disulfide bridges between the chains and are classified into high-molecular-weight (HMW) and low-molecular-weight (LMW) gluten subunits, after the process of reduction and separation using SDS-PAGE [22]. Wheat gluten contains approximately equal amounts of gliadins and glutenins.

Studies have been conducted on IgE reactivity of patients with various clinical profiles of wheat allergy (food allergy, WDEIA, anaphylaxis and baker's asthma) to salt-soluble and insoluble fractions of wheat flour proteins [23]. A high degree of heterogeneity was found in the recognition pattern of allergens among the patient groups with different clinical profiles, as well as within each group. Generally speaking, however, salt-soluble proteins are associated more with flour-inhaled asthma, while prolamins are associated more with WDEIA, and both protein fractions react with the IgE of patients with typical immediate-type wheat food allergy.

### The family of alpha-amylase/trypsin inhibitors

Alpha-amylase/trypsin inhibitors are the main family of wheat allergens responsible not only for respiratory allergy but also for food allergy [21,22,24]. This family constitutes a large part of salt-soluble wheat flour proteins, including the proteins known as CM, which can be extracted with chloroform/methanol mixtures. Three types of alpha-amylase inhibitors have been identified in wheat flour based on their polymeric structure: monomeric or WMAI (one subunit), homodimeric or WDAI (2 identical subunits), and heterotetrameric or WTAI (three different subunits, one of them in 2 copies) [22].

The pioneer study by James et al [25] showed the involvement of WDAI-1 in food allergy to wheat in pediatric patients. Several studies have subsequently identified inhibitor subunits as relevant IgE-binding proteins using sera from allergic patients [26-29], and their *in vivo* reactivity has been demonstrated [30].

Pastorello et al [24] confirmed the role of this family of allergens in food allergy to wheat. These authors found that more than 70% of the sera of 22 European patients



with allergic reactions to food containing wheat presented specific IgE to the subunits of the inhibitors WDAI-2, WTAI-CM1, -CM2, -CM3, and/or -CM16. Most patients with baker's asthma (87%) caused by wheat flour react to a preparation of proteins rich in inhibitor subunits, as well as to at least one isolated subunit of inhibitors (80%). However, the positive responses to purified inhibitors tested so far have ranged from 16% at 45%, with one subunit of the glycosylated tetrameric inhibitor (gWTAI-CM16) being the most reactive allergen protein [31,32].

### **Peroxidase**

Sánchez-Monge et al [33] isolated a protein of 36 kDa with considerable ability to bind IgE from diploid wheat flour (*Triticum monococcum*). The sera from 6 of 10 patients with baker's asthma showed *in vitro* reactivity (dot-blot) to this purified allergen. Wheat peroxidase has also been involved in protein contact dermatitis [16] and in food allergy [24].

### **Thioredoxin**

Wheat thioredoxin, known as Tri a 25, has been described as a new allergen related to flour inhalation asthma [34] and has also been implicated in wheat food allergy [27]. Thioredoxins are ubiquitous regulatory proteins of 12–14 kDa, which reduce the intrachain disulfide bridges of target proteins, such as the wheat storage prolamins (gliadins and glutenins), thereby improving the mobility of these storage proteins in germinating wheat seeds [35].

### **Nonspecific lipid-transfer proteins**

Vegetable lipid-transfer proteins (LTPs) constitutes a family of panallergens consisting of basic polypeptides of 9 kDa (90–95 amino acid residues) that exhibit a three-dimensional structure characterized by a compact domain composed of 4 alpha helices strongly bound by 4 disulfide bridges that belong to the pathogenesis-related (PR) protein family PR-14 [36]. LTPs have been identified as the main allergens in many vegetable foods, mainly Rosaceae fruits (peach, apple, etc.), and in a number of pollens [36-38].

Wheat flour LTP, known as Tri a 14, has been identified as an important food allergen involved in IgE-mediated reactions caused by consuming wheat-derivative products [24,39,40]. Palacin et al [41] also characterized Tri a 14 as a main allergen among Spanish baker's asthma patients. Recombinant Tri a 14 has been produced, and its physicochemical properties, resistance to heat and to proteolysis and its ability to bind to IgE are almost equivalent to those of its natural homolog [42].

### **Serine protease inhibitors**

Constantin et al [43] identified a serine protease inhibitor (Tri a 39) as a new allergen in baker's asthma. This allergen is a 9.9 kDa protein, which generally forms 40 kDa tetramers, which represent a new member of the potato inhibitor I family. The inhibitor is mainly expressed in mature grains, accumulating in the endosperm and in the aleurone layer. The inhibitor is probably involved in plant defense and belongs to the family of pathogenesis-related proteins (PR)-6.

The recombinant form of this allergen reacted with the specific IgE of 3 (14%)[43] and 6 (27%) [44] sera of 22 Spanish baker's asthma patients, when analyzed with dot-blot and microarray, respectively. In contrast, this inhibitor was not recognized by the sera of patients with food allergy to wheat or to grass pollen [43,44].

Wheat serpin (Tri a 33) which belong to the superfamily of SERine Protease Inhibitors have also been shown to be involved in both food and respiratory wheat allergies [24,29,45]. More recently, Sander et al [46] developed ImmunoCAPs with one recombinant serpin which was found to bind IgE in 5% of 28 sera from baker's asthma patients. Mameri et al [47] evaluated the allergenicity of serpin (Tri a 33) allergenicity

using sera from 103 patients with food allergy and 29 patients with baker's asthma. Twenty percent of patients with wheat food allergy and 31% of those with baker's asthma had specific IgE to recombinant serpin, and this allergen was able to induce IgE-dependent basophil degranulation. Epitope mapping identified four regions involved in IgE binding to serpin.

### **Thaumatococcus-like proteins**

Thaumatococcus-like proteins (TLPs) constitute the latest family of saline-soluble wheat flour proteins that have been associated with respiratory allergy to flour [48], as well as with wheat food allergy [49]. Most TLPs have a molecular mass that varies between 21 and 26 kDa and contain 16 preserved cysteine residues that form 8 disulfide bridges responsible for a compact three-dimensional structure and resistance to low pH conditions, proteolysis and heat treatment [50,51].

Purified wheat TLP induced positive skin responses in 6 (30%) and 9 (45%) of 20 Finnish patients with baker's asthma [48].

### **Alpha-purothionin**

Pahr et al [52,53] have described a new wheat food allergen,  $\alpha$ -purothionin (Tri a 37), that is associated with severe allergy.  $\alpha$ -Purothionin is a salt-soluble wheat allergen that can be found in the albumin/globulin fraction. This allergen belongs to the pathogenesis-related protein (PR)-13 family, which has been shown to lyse membranes of attacking pathogens.

IgE reactivity to this allergen has been found to be highly specific for wheat food allergy (positive sera in 11% to 23% wheat allergic European patients) since patients allergic to grass pollen did not show IgE reactivity, and only 3% of patients with baker's asthma showed IgE binding to this allergen [52]. rTri a 37 was frequently recognized by patients with severe anaphylactic reactions to wheat and it has been proposed that could represent a diagnostic marker for an increased risk of anaphylaxis.

### **Prolamins: gliadins and glutenins**

Several of the main insoluble wheat flour proteins (prolamins) are involved in food allergy to wheat, as well as being involved in some cases of respiratory allergy to flour. Studies conducted by Walsh et al [54], Sandiford et al [55] and Mittag et al [56], among others, demonstrated IgE binding in the prolamin fraction, including alpha, beta, gamma and omega gliadins, as well as to LMW glutenin subunits [55]. However, few studies have so far evaluated the allergenicity of recombinant purified prolamins [57-60].

Snegaroff et al [57] produced 2 recombinant LMW glutenin subunits designated LMW-GS B16 (37.7 kDa) and LMW-GS P73 (32.4 kDa). Both glutenin subunits recognized specific IgE from patients with WDEIA or with IgE-mediated allergy to hydrolyzed wheat proteins.

Baar et al [59] characterized Tri a 36, a novel food allergen that belongs to the LMW glutenin subunits, which can be found in a variety of cereals. Recombinant Tri a 36 was recognized by the serum IgE of approximately 80% of the patients with food allergy to wheat, showing cross-reactivity with related allergens in rye, barley, oat and rice, and induced the dose-dependent activation of basophils. In a study in 37 children with wheat food allergy, 21 out of the 37 (57%) patients with wheat food allergy and only 1 patient with grass pollen allergy (7%) showed IgE reactivity to Tri a 36 [60]. Therefore, Tri a 36 is a main food allergen in wheat, which can be used for molecular diagnosis. Prolamins, especially omega-5 gliadin, have been involved in food allergy to wheat and in the WDEIA [13]. WDEIA is generally associated with sensitization to the main wheat allergen omega-5 gliadin (Tri a 19) [61], whose IgE-binding epitopes have been identified [62]. The measurement of serum IgE antibodies against recombinant omega-5 gliadin has been proposed as a marker of this syndrome [63], which can be supplemented with detection of specific IgE to the recombinant HMW glutenin subunit

[64]. Nevertheless, other allergens have been implicated in this syndrome, such as gamma and alpha/beta gliadins [65].

Mammeri et al [66] described that patients with cutaneous allergy to HWP frequently reacted with a recombinant omega gliadin-like D-type glutenin and recombinant alpha-gliadin, whereas IgE reactivity was less common in patients with wheat food allergy, and sera from patients with WDEIA recognized recombinant omega-5 gliadin-like D-type glutenin. Interestingly, it has been reported that there are differences in IgE-binding epitopes between patients with food allergy to wheat. Specific IgE from patients suffering from urticaria and anaphylaxis or WDEIA detected sequential epitopes in the repetitive domain of gliadins, whereas IgE from atopic dermatitis patients seem to recognize conformational epitopes [67].

The levels of specific IgE to omega-5 gliadin correlated well with the results of oral provocation in Japanese children with wheat allergy [11,68]. More than 80% of the children with positive provocation to wheat had specific IgE against omega-5 gliadin, while this was not detected in any of the children with negative oral provocation [11]. These results have been corroborated in a multicenter study with a pediatric population in which wheat allergy was confirmed by oral challenge [69]. Also, 72% of the children with wheat allergy had positive specific IgE to omega-5 gliadin, while 75% of the children with no allergy had negative specific IgE. There was a significant correlation between the probability of having wheat allergy and the serum concentration of IgE to omega-5 gliadin, especially in children younger than 1 year of age [69]. In contrast, Beyer et al [70] found no relationship between levels of specific IgE to omega-5 gliadin and the results of oral provocation in German and American children with suspected food allergy to wheat.

### Other allergens

Bittner et al [71] have recently identified 6 wheat proteins that bound IgE in the sera of patients with diagnosed baker's asthma. Three of them are known plant transcription factors, one protein is a translation factor, and 2 of the proteins are uncharacterized. Buckwheat (*Fagopyrum esculentum*), although not a cereal, can be found in numerous processed foods (pasta, pizza, snacks), often as a hidden allergen [72].

Respiratory allergy to lupine (*Lupinus albus*) has been described after occupational exposure, and it has been shown that one third of 116 symptomatic bakers showed specific IgE to lupine. At least some of these sensitizations were based on cross-reactivity between lupine and wheat flour [73].

Other issues to consider include additives and contaminants that can be present in cereal flour. For example, the oral mite anaphylaxis (pancake syndrome) characterized by severe symptoms caused by ingestion of foods made with wheat and corn flour, especially pancakes, that are contaminated by domestic or storage mites [74].

A summary of wheat allergens can be found in table 1.

### DIAGNOSIS

SPT have a very important role in the diagnosis of IgE-mediated allergy to cereals, as with other foods. Nevertheless, the reliability of skin tests is closely related to the quality, potency and standardization of the allergenic extracts, which often are ill-defined in commercial cereal extracts.

Sander et al [75] conducted a study to compare the various wheat and rye flour extracts used for SPT. The wheat and rye flour extracts from 3 different companies differed in the concentration and composition of proteins, which resulted in considerable variability in SPT results (applied to respiratory allergy to cereal flour). These authors also found that the sensitivity of specific IgE measurements (by ELISA or ImmunoCAP) was superior to those of SPT with commercial wheat and rye extracts. The diagnosis of grain-induced asthma has been thoroughly reviewed [7].

In the case of food allergy to cereals, SPT or specific IgE determinations with only the salt-soluble fraction had poor predictive values. It is also necessary to perform SPT or measurements of specific IgE to gliadin (of the salt-insoluble fraction), which constitute very important additional tools for studying wheat allergy by ingestion [10,11].

The diagnosis of cereal allergy will therefore depend on the presence and concentration of the main allergens in the extracts used for diagnostic tests. The identification and characterization of wheat allergens is therefore crucial [76].

We also must consider that cereals are part of the *Gramineae* family, and there is extensive allergen cross-reactivity between wheat flour and grass pollen [77], which can complicate the diagnosis, particularly when IgE binds to cross-reactive carbohydrate determinants [78]. Patients with grass pollen allergy sometimes have positive SPT and specific IgE tests to wheat flour, but the development of symptoms by ingesting cereals is exceptional in these individuals [79].

The basophil histamine release test has also been employed to study allergy to several foods, including wheat flour. Sato et al [80] compared the positivity threshold of the histamine release test with the results of the oral challenge with wheat and established that the optimal cutoff was 500 ng/ml of wheat antigen, with a diagnostic efficiency of 70.7%.

Oral provocation with wheat or the implicated cereal is the reference test for the definitive diagnosis of ingested wheat/cereal allergy. Oral food challenges are usually required to confirm the diagnosis of food allergy, to monitor food allergy, or to prove oral tolerance to a given food [8]. Although the double-blind placebo-controlled food challenge (DBPCFC) is considered the gold standard test for the diagnosis of food allergy, in many cases, an open oral food challenge with an objective reaction is sufficient for the diagnosis of food allergy [8]. Using the DBPCFC with wheat as the reference standard, a systematic review found pooled sensitivities for SPT and specific IgE of 73% (95% Confident Interval 56-85) and 83% (95% CI 69-92), and specificities of 73% (95% CI 48-89) and 43% (95% CI 20-69%), respectively [81].

Thus, it is important to keep in mind that oral food challenge is the gold standard for diagnosis of food allergy, including wheat allergy.

There are scarce data on the *in vivo* cross-reactivity among the various cereals. By using the oral challenge test with various cereals in a pediatric population, studies have reported that 80% of children with allergy to cereals react to only one type of cereal. Prohibiting all cereals is therefore not justified [79].

Mäkelä et al [82] carried out a study among 118 children with suspected wheat food allergy to evaluate the wheat allergens with higher efficiency for predicting clinical reactivity using DBPCFC oral wheat challenges. Thirty (28%) children experienced immediate symptoms, 27 (25%) delayed symptoms to ingested wheat, and 51 (47%) children had not reactions in oral wheat challenges. The allergenic components of wheat that better discriminated those with immediate symptoms from those who did not experience any reactions were alpha-amylase inhibitors, in particular dimeric alpha-amylase inhibitor 0.19, alpha-, beta-, and gamma-gliadins, and HMW glutenin subunits. Diagnosis of WDEIA is made based on the patient's history in combination with allergy skin testing, determination of wheat-specific IgE serum antibodies, basophil activation test, histamine release test, and/or exercise challenge test [13]. Notwithstanding, an oral challenge with gluten alone or along with aspirin and alcohol has been shown to be a sensitive and specific test for the diagnosis of WDEIA, and exercise does not seem to be an essential trigger for the onset of symptoms in patients with WDEIA [83]. For patients with wheat allergy caused by a specific HWP (Glupearl 19S), usually manifested as a variant form of WDEIA, the SPT is considered to be the most effective diagnostic method, and an ELISA-based GP19S-specific IgE assay has been recently validated [84].

## Component-resolved diagnosis

Microarray assays with recombinant allergens of wheat flour and grass pollen have been successfully used to discriminate and refine the diagnosis of asthma by wheat flour inhalation, wheat food allergy and allergy to grass pollen [44].

Pahr et al [85] used molecular diagnosis with five recombinant wheat allergens (a thioredoxin h isoform, glutathione transferase, 1-Cys-peroxiredoxin, profilin and dehydrin) to discriminate different clinical manifestations of wheat allergy (respiratory vs food allergy). 1-Cys-peroxiredoxin, glutathione transferase and dehydrin were mainly recognized by patients with baker's asthma but not by patients with wheat food allergy.

Nilsson et al [86] carried out a study on 63 children with a doctor's diagnosis of wheat allergy and allergy to wheat was confirmed in 32 of them (26 were positive in challenge and 6 were regarded as wheat allergic due to recent anaphylactic reactions). Specific IgE antibodies to  $\omega$ -5 gliadin, LMW-glutenin, HMW-glutenin and a native gliadin preparation containing alpha-, beta-, gamma, and omega-gliadin were determined. The levels of specific IgE to all four wheat allergens were significantly higher in the group with wheat allergy compared to the group with no wheat allergy. The severity of symptoms during the challenge correlated with the levels of specific IgE to all four allergens, and the best correlation with challenge outcome was found with specific IgE to omega-5 gliadin.

These results reinforce the importance of oral provocation test as the gold standard for the diagnosis of wheat allergy, although the employment of component-resolved diagnosis, meaning the combination of different sIgE to wheat-components, can improve the positive predictive value of other diagnostic tests.

With regard to occupational allergy to wheat flour, Olivieri et al [87] conducted a study of 81 bakers reporting work-related symptoms using commercially available SPT, specific IgE, and ISAC microarray tests as well as six additional dot-blotted wheat allergens (Tri a39, Tri a Trx, Tri a GST, Tri a32, Tri a12, Tri a DH). It was found Tri a 30, Tri a 32 and Tri a GST allergens were associated with work-related dermatitis.

Gómez-Casado et al [88] characterized the sensitization profile of 45 Spanish baker's patients using a panel of 12 wheat allergens (WDAI-0.19, WDAI-0.53, WTAI-CM1, WTAI-CM2, WTAI-CM3, WTAI-CM16, WTAI-CM17, Tri a 14, profilin,  $\omega$ -5-gliadin, Tri a Bd 36 and Tri a TLP) that were purified and printed on a wheat-allergen microarray. A group of subjects with seasonal allergic rhinitis due to grass pollen (SAR) and wheat food allergy were also analysed for comparison. WTAI-CM16 and Tri a 14 were identified as the most prevalent allergens (sensitization rate 54% and 45%, respectively), being positive in 64% of the BA population. By contrast,  $\omega$ -5-gliadin and Tri a Bd 36 were recognized by less than 10% of the baker's population. Tri a 14 (wheat lipid transfer protein) was exclusively recognized by baker's asthma patients (44%) and not by patients with wheat food allergy or SAR patients.

Sander et al [89] evaluated IgE reactivity to wheat allergen components in patients with baker's asthma as well as the potential cross-reactivity with grass pollen. Nineteen recombinant wheat flour allergens and 2 cross-reactive carbohydrate determinants were tested by using commercial IgE tests in sera of 101 European bakers allergic to wheat flour and 29 control subjects sensitized to pollen who had specific IgE to wheat but no occupational exposure. Eighty percent of bakers had positive specific IgE to at least one of the 21 allergens. The highest frequencies of IgE binding were found for thiol reductase (Tri a 27) and the wheat dimeric  $\alpha$ -amylase inhibitor 0.19 (Tri a 28). Cross-reactivity to grass pollen was observed for 9 components. A combination of IgE tests to 5 allergens, Tri a 27, Tri a 28, tetrameric  $\alpha$ -amylase inhibitor CM2 (Tri a 29.02), serine protease inhibitor-like allergen (Tri a 39), and 1-cys-peroxiredoxin (Tri a 32), showed the maximal area under the curve (AUC = 0.84), but this was still lower than the AUC for wheat- or rye flour-specific IgE (AUC = 0.89 or 0.88, respectively). It was concluded that component-resolved diagnostics help to distinguish between sensitization caused by occupational flour exposure and positive serologic IgE tests to



wheat flour due to cross-reactivity with grass pollen. Thus, for clinical diagnosis of baker's asthma, specific IgE tests with whole wheat and rye flour extracts remain the test of choice due to its superior diagnostic sensitivity.

## MANAGEMENT

For now, the only treatment for food allergy to cereals is eliminating them from the diet and strict avoidance, while waiting for tolerance to occur spontaneously, should it occur at all. There are some preliminary data on the possibility of specific oral tolerance induction (SOTI) to wheat through desensitization protocols in children [90-92], but this is still considered an experimental treatment.

Rodríguez del Río et al [91] investigated the safety and efficacy and of a SOTI protocol with wheat in the treatment of wheat allergy. Six wheat allergic children assessed by a DBPCFC underwent wheat SOTI with an up-dosing phase until 100 g of wheat was tolerated, followed by a 6-month maintenance phase. Specific IgE to wheat and other cereals as well as specific IgE, IgG4, and IgG1 to a panel of isolated wheat proteins (alpha-amylase and trypsin inhibitors, LTP, gliadins, and glutenins) were measured. Threshold doses in the wheat DBPCFC ranged from 6.6 g to 96.6 g. Five out of 6 patients successfully finished the up-dosing phase in 3 to 24 days. The protocol was well tolerated since only 6.25% of doses in the up-dosing phase elicited mild adverse reactions. After a 6-month maintenance phase, all the patients showed good tolerance of 100 g of wheat daily. The 5 patients who successfully finished the up-dosing phase also tolerated rye after SOTI, and all but 1 tolerated oat as well. None of the patients showed specific IgE to omega-5-gliadin, whereas alpha-amylase inhibitors were recognized by all patients. Specific IgG4 and IgG1 increased in all patients. The utility of SOTI with wheat has been corroborated by other authors in two children allergic to gluten and/or omega-5-gliadin [92].

For patients with respiratory allergy to wheat flour, early diagnosis and early avoidance of further exposure are the cornerstones of management [7]. Whenever feasible the patient should be relocated to a job category without exposure. Pharmacological treatment of occupational asthma should comply with published asthma guidelines.

Omalizumab has been shown to have clinical benefit in a few patients with uncontrolled severe baker's asthma [93-95].

Subcutaneous immunotherapy with wheat flour extracts has been tested in baker's asthma with favorable results in small studies [96,97], and its role in the treatment of occupational asthma has not yet been defined.

## EXPERT COMMENTARY

The clinical presentation of cereal hypersensitivity in general and wheat allergy in particular has many faces and challenges the practicing clinician.

Wheat allergy diagnostics is difficult, even using sophisticated component methods.

For the diagnosis is crucial to consider the diversity of specific phenotypes of wheat hypersensitivity (inhaled or food allergy, anaphylaxis associated with cofactors, and non-allergic sensitivity). The accuracy of IgE-mediated wheat allergy diagnosis may be improved by measuring IgE responses to several components of wheat. However, a high degree of heterogeneity has been found in the recognition pattern of allergens among patient groups with different clinical profiles, as well as within each group, which makes difficult the clinical interpretation and applicability of component-resolved diagnosis. It is promising that IgE reactivity to some wheat allergens has been associated with specific phenotypes (e.g. WDEIA) and with severe anaphylactic reactions to wheat. Thus, identifying sensitization to these allergens may represent a diagnostic marker for an increased clinical severity and a higher risk of anaphylaxis. However, it should be stressed that the oral food provocation testing is the gold standard for the diagnosis of food allergy, including wheat allergy.



The use of new therapeutic approaches, such as allergen immunotherapy and biologics for severe respiratory allergy to wheat flour, as well as specific tolerance induction for persistent wheat food allergy, is under evaluation and positive preliminary results have been already published.

## FIVE-YEAR VIEW

There are still marked limitations in the diagnosis and treatment of allergy to cereals. The identification and characterization of cereal allergens, in particular those of wheat flour, which is associated with various clinical allergy profiles, should enable us to better understand the role played by major and minor allergens, and can help us create an appropriate diagnostic panel of molecular markers.

Furthermore, they can contribute to: 1) establishing potential associations between sensitization profiles and clinical symptoms, geographical areas or patient ages; 2) comparing the compounds involved in various sensitization pathways (ingestion vs. inhalation); 3) predicting potential cross-reactions between vegetable food allergens and pollen allergens; 4) researching changes in the allergen capacity of cereal foods (wheat); and 5) designing allergenic variants with modified properties (i.e., less ability to bind to IgE).

Our knowledge of the wheat allergens related to food and respiratory allergies has, at least, the following limitations: 1) wide variability in the clinical and immunological characteristics of the patients included in most studies, which frequently have a small sample size, making it difficult to generalize the findings; 2) many of the purified allergens show a low prevalence of IgE recognition (with the exception of gliadins in some clinical contexts, such as WDEIA) which could be partly explained by the considerable individual heterogeneity in the sensitization patterns; 3) many studies report only *in vitro* IgE reactivity results to wheat allergens, whereas few studies include *in vivo* diagnostic procedures and challenge tests; 4) scarce information pertaining clinical data on the symptoms, sensitization pathway, and oral tolerance of wheat products among patients with respiratory allergy; 5) limited comparative studies between natural and recombinant allergens; 6) an unknown role of N-linked glycan complexes (CCD); and 7) no conclusive results on the cross-reactivity with other cereal flours (rye, barley, oat), vegetable foods and pollens (particularly grass pollen). Therefore, the molecular diagnosis of wheat and cereal allergy is still in a preliminary (although promising) phase and requires further basic and clinical research.

## KEY ISSUES

- IgE-mediated food allergy to wheat and other cereals has a low prevalence in Western countries whereas respiratory allergy to cereal flour is relatively common among exposed workers.
- Wheat food allergy in children is usually associated with a good prognosis and can be outgrown.
- Some patients with wheat allergy can develop severe allergic reactions, including anaphylaxis.
- Several wheat allergens have been identified and may be useful for the development of serological tests which allow the discrimination of different clinical manifestations of wheat allergy.
- Wheat omega-5 gliadin is a major allergen associated with WDEIA, and detection of circulating specific IgE to recombinant omega-5 gliadin is a reliable method for its diagnosis.

- Currently, the cornerstone in the treatment of food allergy is avoidance of the incriminated food until outgrowing the allergy. However, specific oral tolerance induction with wheat is being investigated.
- For patients with respiratory allergy to wheat flour the treatment of choice is avoidance of exposure and relocation of affected workers to areas with no exposure to flour.
- Allergen-specific immunotherapy and biological treatments, such as anti-IgE monoclonal antibodies (omalizumab), may play a role in management of highly selected patients.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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*\* Of interest*

*\*\* Of considerable interest*

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Table I. Wheat allergens (adapted from WHO/IUIS Allergen Nomenclature Sub-Committee) (accessible at <http://www.allergen.org/>)

Allergen	Biochemical name	MW (kDa)	Food allergen
Tri a 12	Profilin	14	Yes
Tri a 14	Non-specific lipid transfer protein 1	9	Yes
Tri a 15	Monomeric alpha-amylase inhibitor 0.28	13.2	No
Tri a 18	Agglutinin isolectin 1	21.2	Yes
Tri a 19	Omega-5 gliadin	65	Yes
Tri a 20	Gamma gliadin	35-38	Yes
Tri a 21	Alpha-beta-gliadin	32.6	No
Tri a 25	Thioredoxin	13.3	Yes
Tri a 26	High molecular weight glutenin	88	Yes
Tri a 27	Thiol reductase homologue	27	No
Tri a 28	Dimeric alpha-amylase inhibitor 0.19	13	Yes
Tri a 29	Tetrameric alpha-amylase inhibitor CM1/CM2	13	Yes
Tri a 30	Tetrameric alpha-amylase inhibitor CM3	16	Yes
Tri a 31	Triosephosphate-isomerase	26.8	No
Tri a 32	1-cys-peroxiredoxin	23.9	No
Tri a 33	Serpin	43.3	No
Tri a 34	Glyceraldehyde-3-phosphate-dehydrogenase	36.5	No
Tri a 35	Dehydrin	11.5	No
Tri a 36	Low molecular weight glutenin GluB3-23	40	Yes
Tri a 37	Alpha-purothionin	12	Yes
Tri a 39	Serine protease inhibitor-like protein	9.9	No
Tri a 40	CM 17 protein (alpha-amylase inhibitor)	15.9	Yes
Tri a 41	Mitochondrial ubiquitin ligase activator of NFKB 1	6.9	Yes
Tri a 42	Uncharacterized protein	8.2	Yes
Tri a 43	Uncharacterized protein	11.6	Yes
Tri a 44	Endosperm transfer cell specific PR60 precursor	11.9	Yes
Tri a 45	Elongation factor 1 (EIF1)	9.7	Yes

CM: Chloroform/methanol-soluble