

Clinical Features of Wheat Allergy Are Significantly Different between Tri a 14 Sensitized Patients and Tri a 19 Sensitized Ones

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Keywords

Wheat allergy · Omega-5-gliadin · Lipid transfer proteins · Food allergy · Wheat-dependent exercise-induced anaphylaxis

Abstract

Introduction: Wheat is the most important cereal for human nutrition but its high consumption is associated to an increasing complaint of wheat-related disorders, many of which are allergic in nature and different in respect to the involved allergens. In this study, we compared the clinical aspects of wheat allergy presented by patients sensitized to Tri a 19 in respect to those presented by patients sensitized to Tri a 14. **Methods:** With this aim, we selected patients sensitized to 1 or both of the 2 allergens, and among these we identified those who were really wheat allergic and reactive on the basis of a standardized methodology. We evaluated the clinical features such as the kind and severity of symptoms, the coexistence of triggering factors such as physical exercise and NSAIDs and alcohol consumption, and the association with other allergens and with various immunologic parameters. Wheat allergy in Tri a 19 sensitized patients was confirmed

through a questionnaire while the patients sensitized to Tri a 14 underwent wheat challenge with 100 g of pasta followed by exercise on a treadmill. **Results:** Seventy-nine patients sensitized to Tri a 14 and 40 patients sensitized to Tri a 19 were recruited. The 2 sensitizations were independent with a significant inverse relation ($p < 0.00001$). The Tri a 19 sensitized patients presented, in respect to the Tri a 14 sensitized ones, an older age ($p = 0.0017$), a higher risk to be wheat allergic ($p < 0.0001$), a higher severity of the reactions ($p < 0.00001$) and a higher association with some cofactors, namely alcohol ($p < 0.0005$) and physical exercise ($p = 0.003$). On the contrary, Tri a 14 sensitization was associated with atopy ($p < 0.0001$), with a higher probability of patients being asymptomatic ($p < 0.0001$) and being sensitized to other foods, in particular to nuts and cereals ($p < 0.00001$). **Conclusions:** Sensitization to Tri a 19 or Tri a 14 determines different clinical pictures. In particular, sensitization to Tri a 19 implies a higher probability of severe reactions, even dependent on daily triggers, while that to Tri a 14 implies a higher cross-reactivity with other foods but it's more frequently asymptomatic, making a food challenge necessary to prevent useless food avoidance.

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Introduction

Wheat is the most important staple crop for human nutrition, and it is in increasing demand worldwide with important wheat-yield estimates, especially for soft wheat [1]. The increase in demand and production, however, has given way to an increase in the number of reported wheat-related disorders, and this may eventually hamper wheat production and distribution in the future. In fact, an increase in their incidence has been significantly correlated with the increased consumption of this cereal [2]. Modern wheat, compared to that cultivated in the past, has a higher content of gluten and immunogenic epitopes as a result of current cultivation practices and varietal selection [3, 4]. This could partly be the cause of this epidemiological variation. Among wheat-related diseases, apart from the traditionally considered ones such as coeliac disease, baker's asthma, IgE-mediated food allergy, and the emerging eosinophilic esophagitis, other non-immunological conditions such as irritable bowel syndrome and gluten sensitivity have become a reason for concern, inasmuch as they are associated with the mystified belief that "gluten free" is synonymous with "health products." Thus, since wheat is of paramount nutritional importance, it is essential to develop effective diagnostic criteria to investigate all the suspected wheat-related disorders and avoid generic labels of allergy or intolerance (a term that has been exploited but has no clear clinical significance), presently widespread and potentially harmful. Concerning food allergic reactions, wheat can induce a classical clinical picture of urticaria/angioedema and anaphylaxis or a severe form of exercise-related wheat allergy (wheat-dependent exercise-induced anaphylaxis (WDEIA)). To date, 28 allergens of *Triticum aestivum* (soft wheat) have been described, which are variously associated with different clinical situations. The most relevant major allergens among these are the following: α -amylase inhibitor, lipid transfer protein (LTP), and omega-5-gliadin. Sensitization to wheat LTP, Tri a 14, is prevalent in the Mediterranean area and is associated with sensitization to Pru p 3, the LTP of peach, albeit with low frequency [5]. Sensitization to this protein correlates with the manifestations of classical food allergy but also with WDEIA and baker's asthma [6, 7]. Recently, the LTP of durum wheat, Tri tu 14, has also been sequenced [8] and seems to be strongly associated with food allergy. The omega-5-gliadin, Tri a 19, a fraction of gluten, is considered the major allergen of WDEIA [9]. More recently, other allergens such as alpha-, beta-, and gamma-gliadins, as

well as high and low molecular weight glutenins, have also been associated with this condition [10, 11]. In particular, molecular assays are commercially available for omega-5-gliadin, LTP, and α -amylase inhibitor, making IgE detection much more sensitive and specific, allowing the study of selected case series in order to compare the clinical features affected by sensitizations to each allergenic profile. While sensitization to these proteins seems to be associated with different clinical manifestations, other aspects remain unknown, such as their clinical courses in terms of severity and evolution. In a case series of subjects with classical wheat allergy demonstrated by double-blind placebo-controlled food challenge (DBPCFC) and with a prevalent sensitization to α -amylase inhibitor and LTP, we reported a favourable prognosis with reduction of clinical manifestations due to wheat a few years after diagnosis [12]. Interestingly, in a recent work, Hamada demonstrated the persistence of baseline values of omega-5-gliadin specific IgE in subjects sensitized to this protein even several years later, although without investigating the clinical behaviour [13]. All these data seem to demonstrate a certain degree of association between the positivity to some wheat allergens and the natural history of the clinical picture. Our study addressed the clinical aspects of WDEIA induced by sensitization to Tri a 14 or to Tri a 19, looking in particular at the possible differences conditioned by the 2 kinds of sensitization in regard to symptoms and cross-reactivity with other foods.

Materials and Methods

Patient Selection

An observational study was conducted on 124 adult patients referred to the Department of Allergology and Clinical Immunology of the ASST Grande Ospedale Metropolitano Niguarda (Milan, Italy) for any kind of food allergic reactions and IgE positivity for Tri a 19 and/or Tri a 14. In the selected patients, we investigated the features of the suspected food reactions, classified according to the Müller grading system, the possible role of wheat and the history of respiratory allergies. Besides, a possible relationship between suspected allergic reactions to food with exercise, alcohol consumption, and NSAID intake was evaluated. In all selected patients, specific IgE levels for food, such as rice, maize, barley, oats, rye, peanut, walnut, hazelnut, almond, shrimp, and peach LTP, Pru p 3, and inhalant allergens, such as *Phleum pratense*, *Dermatophagoides pteronyssinus*, and birch were reported.

The diagnosis of wheat allergy in the selected patients was made according to different protocols for Tri a 19 positive and Tri a 14 positive patients. Wheat allergy in Tri a 19 positive patients was diagnosed as suggested by Jiang et al. [14] based on a documented clinical history of anaphylaxis or at least of a systemic reaction as resulted from the answers to a published questionnaire.

Moreover, we proposed to these patients to verify the symptomatology after ingestion of a standard dosage of the suspected wheat-containing product plus exercise. Thus, when accepted, we performed a wheat challenge test with 100 g of pasta plus exercise considering this amount sufficient.

Wheat allergy among Tri a 14 positive patients was more complex to verify as in several cases there was no suspicion of wheat allergy as other vegetable foods determined the reactions. In most of the cases with a suspected wheat allergy, it was necessary to perform wheat challenges such as open challenges and/or DBPC wheat challenge with associated exercise when WDEIA was suspected, except for those patients with a documented clinical history of a severe systemic IgE-mediated reaction.

Challenge Procedures

Double-Blind Placebo-Controlled Food Challenge

A DBPCFC with 50 g raw wheat flour was performed on all patients. In the case of a positive raw wheat DBPCFC, the patient also underwent cooked wheat DBPCFC. Wheat was eliminated from the diet at least 1 week before the challenge. The patients were given the test meals as inpatients on separate days after an overnight fast. The ingredients used for their preparation and the methodology for the clinical procedure were the same used in our previous studies [15].

Exercise-Wheat Challenge

In order to verify the appearance of symptoms after a standard amount of the suspected food after exercise, we conducted a challenge with 100 g of cooked wheat pasta and a 30-min run on the treadmill. For the methodology, we specified that the speed and progression of the run were tailored according to the patients' baseline fitness preparation; the goal was to achieve and maintain a cardiovascular frequency range 120–160 bpm, according to age.

Immuno CAP

An immunoCAP assay (Thermo Scientific, Uppsala, Sweden) was applied to measurement of the levels of total serum IgE and serum specific IgE for inhalants, foods, and recombinant allergens. A positive result of specific IgE values was considered >0.10 KU/L.

Results

Patients and Demographic Features

We identified 79/124 patients sensitized to Tri a 14, 40/124 patients sensitized to Tri a 19 and 5/124 patients sensitized to both Tri a 14 and Tri a 19. Patients sensitized to both allergens were excluded from the final data analysis. We observed a significant difference in the distribution of sex and age in the 2 groups. Males were more frequently Tri a 19 positive. A total of 35/79 (44.3%) Tri a 14 sensitized patients were male versus 26/40 (65%) among those sensitized to Tri a 19. Tri a 19 sensitized patients had a mean age of 48 ± 17.2 years being significantly older than those Tri a 14 sensitized, who showed

a mean age of 37.9 ± 12.5 years (Welch test, $p = 0.0017$). Concerning atopy, 67.1% (53/79) of the Tri a 14 sensitized patients were atopic in respect to 32.5% (13/40) of the Tri a 19 sensitized ones (Fisher's exact test, $p < 0.0001$).

Wheat Clinical Allergy in Tri a 14 and Tri a 19 Sensitized Patients

The clinical association between Tri a 14 or Tri a 19 specific IgE levels with the presence of symptoms for wheat, was evaluated by comparing the data collected either from clinical history or from the challenges with the "in vitro" results. So, we found that 35/79 (44.3%) Tri a 14 sensitized patients had no history of clinical wheat reactivity and underwent IgE determination for Tri a 14 on the basis of a known LTP syndrome diagnosed for allergy to peach and sensitization to Pru p 3. Among the other 44/79 Tri a 14 sensitized patients, 15/79 (18.9%) have reported severe and documented wheat-induced reactions, while 29/79 (36.7%) patients have reported a cutaneous allergic reaction after a meal containing wheat among other different foods. These underwent a DBPCFC. Among these, 7/29 (24.1%) patients were positive to the DBPCFC and 22/29 (75.8%) were negative. Therefore, among 79 Tri a 14 sensitized patients, 22/79 (27.8%) were truly wheat allergic (as a summation of 15 patients with a documented clinical history of a severe reaction plus 7 patients with a positive DBPCFC). The demographic, clinical, serological data and challenge results regarding 22 wheat allergic Tri a 14 positive patients are shown in Table 1.

In the group of Tri a 19 positive patients assessed by a questionnaire for the presence of clear symptoms upon wheat ingestion with or without predisposing factors such as exercise, NSAIDs and alcohol intake, we found that the relationship between clinical history and IgE levels was positive in 34/40 (85%) patients who were thus diagnosed as allergic to Tri a 19. Demographic, clinical, serological data regarding 34 wheat allergic Tri a 19 positive patients are shown in Table 2. Interestingly, wheat was the only cereal that induced a reaction in all of these patients, with the exception of 2 who experienced anaphylaxis after also eating spelt.

Some of the 34 Tri a 19 allergic patients did not want to eliminate wheat pasta from the routine diet and accepted a wheat challenge followed by exercise. None of them experienced a reaction and nevertheless all of them had a clear history of wheat reactions associated with physical exercise. Furthermore, most of them had even a history of wheat-related reactions facilitated by either

Table 1. Demographic, clinical, serological data and challenge results of wheat-allergic Tri a 14 positive patients

Pt	Sex	Age, years	Wheat symptoms	Reaction grade	Wheat IgE, kU/L	Tri a19 IgE, kU/L	Grass IgE, kU/L	Wheat DBPCFC	Provocation dose, g	Challenge symptoms
1	M	56	WDEIA	4	0.43	32.80	0.00	Positive	50	U
2	M	38	WDEIA	4	0.84	7.90	n.d.	n.d.	n.d.	n.d.
3	M	56	WDEIA	4	0.00	2.28	0.00	n.d.	n.d.	n.d.
4	F	27	WDEIA	3	0.00	1.60	n.d.	n.d.	n.d.	n.d.
5	F	22	U	1	1.57	4.64	0.69	n.d.	n.d.	n.d.
6	M	63	U	1	1.13	11.60	0.12	n.d.	n.d.	n.d.
7	F	28	WDEIA	4	5.38	5.03	0.50	n.d.	n.d.	n.d.
8	F	23	WDEIA	3	0.49	12.80	0.13	n.d.	n.d.	n.d.
9	M	33	WDEIA	3	0.66	13.30	0.00	n.d.	n.d.	n.d.
10	M	35	WDEIA	4	0.32	2.45	48.40	n.d.	n.d.	n.d.
11	M	6	U/AE	1	1.70	9.57	8.51	Positive	50	OAS/U
12	F	51	ANA	4	48.00	31.60	1.68	n.d.	n.d.	n.d.
13	F	38	AE	1	0.13	0.75	0.11	Positive	25	U/AE
14	M	49	ANA	3	1.68	1.37	21.10	n.d.	n.d.	n.d.
15	F	33	U, GI	2	3.61	6.78	n.d.	Positive	50	GI
16	M	29	ANA	3	2.02	1.62	n.d.	n.d.	n.d.	n.d.
17	M	48	ANA	3	2.49	2.74	n.d.	n.d.	n.d.	n.d.
18	M	23	ANA	3	1.10	2.49	0.62	n.d.	n.d.	n.d.
19	F	32	ANA	2	39.00	0.54	0.54	n.d.	n.d.	n.d.
20	M	33	EoGI	3	5.31	1.26	n.d.	Positive	25	GI
21	M	46	GI	2	3.15	6.68	33.00	Positive	50	GI
22	F	46	Asthma, GI	3	0.99	2.45	0.13	Positive	50	GI, cough

Reaction grade: severity of reaction according to the Müller grading system. F, female; M, male; U, urticaria; WDEIA, wheat-dependent exercise-induced anaphylaxis; AE, angioedema; ANA, anaphylaxis; GI, gastrointestinal symptoms; EoGI, eosinophilic gastroenteritis; OAS, oral allergic syndrome; n.d., not done.

NSAIDs and/or alcohol intake. Thus, we can conclude that Tri a 19 sensitized patients were significantly more prone to develop wheat induced allergic symptoms than Tri a 14 sensitized ones (Fisher's exact test, $p < 0.0001$).

Severity of Reactions

As regards the clinical severity of allergic reactions after wheat ingestion, in the Tri a 14 sensitized patient group, 15/79 (18.9%) had severe systemic reactions such as anaphylaxis, while 7/79 (8.8%) had just mild reactions with cutaneous or gastroenteric manifestations. Among the Tri a 19 patients instead, 22/40 (55%) had severe systemic reactions and 12/40 (30%) had milder symptoms.

Considering the 2 groups of patients, the probability that a patient taken at random in the Tri a 19 sensitized sample had a higher grade reaction than a patient taken at random among Tri a 14 sensitized patients was 80% or vice versa the probability that a Tri a 14 sensitized patient taken at random in the sample had a higher grade reaction than a Tri a 19 positive patient taken at random from the sample is 20% (Mann-Whitney U test, $p < 0.00001$).

Cofactors

Risk factors for allergic reactions to wheat, namely physical exercise, alcohol, and NSAIDs intake, were compared between Tri a 14 and Tri a 19 sensitized patients. A history of exercise-induced anaphylaxis was significantly more frequent in Tri a 19 sensitized patients (80%) than Tri a 14 sensitized ones (15.6%) (Fisher's exact test, $p = 0.003$). A history of NSAID consumption as a trigger of the reaction was reported from 1.3% (1/79) of Tri a 14 sensitized patients and from 7.5% (3/40) of Tri a 19 sensitized patients, without significant differences among the 2 groups (Fisher's exact test, $p = 0.110$). On the contrary, alcohol intake was a cofactor of reaction in 35% (14/40) of patients sensitized to Tri a 19, compared to the only 3.8% (3/79) observed for Tri a 14 sensitized patients, with a highly significant difference (Fisher's exact test, $p < 0.0005$).

Laboratory Results

All enrolled patients had positive wheat-specific IgEs, regardless of sensitization to Tri a 14 or Tri a 19. The me-

Table 2. Demographic, clinical, serological data of wheat-allergic Tri a 19 positive patients

Pt	Sex	Age, years	Wheat symptoms	Reaction grade	Wheat IgE, kU/L	Tri a19 IgE, kU/L	Grass IgE, kU/L
1	M	56	WDEIA	4	0.43	32.80	0.00
2	M	38	WDEIA	4	0.84	7.90	n.d.
3	M	56	WDEIA	4	0.00	2.28	0.00
4	F	27	AE, U	3	0.00	1.60	n.d.
5	F	22	U	1	1.57	4.64	0.69
6	M	63	U	1	0.00	3.21	0.00
7	M	31	U	1	1.33	9.40	87.00
8	M	57	WDEIA	3	0.00	4.87	0.00
9	M	45	U	1	0.52	8.58	0.00
10	M	68	WDEIA	4	0.00	2.90	0.00
11	M	45	WDEIA	4	0.17	10.40	0.57
12	M	56	WDEIA	4	0.92	25.00	0.15
13	F	37	WDEIA	4	0.90	24.10	n.d.
14	M	45	U	1	0.19	6.82	n.d.
15	F	19	U	1	0.16	4.48	n.d.
16	M	46	WDEIA	4	0.23	4.00	n.d.
17	M	71	WDEIA	4	0.63	24.90	0.10
18	F	37	U, A, AE	3	1.13	5.58	0.00
19	M	47	WDEIA	4	n.d.	0.50	18.00
20	F	57	U	1	1.18	10.00	n.d.
21	F	63	WDEIA	4	0.26	2.76	1.57
22	F	59	WDEIA	4	1.66	13.10	0.00
23	M	53	U, AE, GI	3	1.87	38.60	1.25
24	M	51	WDEIA	4	1.18	14.40	n.d.
25	F	50	U, R, AE	1	0.17	4.98	n.d.
26	F	51	U, AE	2	1.94	12.00	0.00
27	M	65	U, A	3	0.70	10.00	n.d.
28	M	34	WDEIA	4	0.97	11.60	0.00
29	M	42	U, AE	1	1.16	12.30	n.d.
30	F	26	WDEIA	4	0.47	10.00	24.60
31	M	61	U	2	1.65	10.20	0.00
32	F	16	U, A, GI, AE	3	0.26	0.20	0.00
33	M	67	U, GI	3	0.56	7.82	0.19
34	F	39	U	1	0.28	81.30	n.d.

Reaction grade: severity of reaction according to the Müller grading system. F, female; M, male; U, urticaria; WDEIA, wheat-dependent exercise-induced anaphylaxis; AE, angioedema; ANA, anaphylaxis; GI, gastrointestinal symptoms; EoGI, eosinophilic gastroenteritis; OAS, oral allergic syndrome; n.d., not done.

dian wheat-specific IgE levels were significantly higher in Tri a 14 positive patients (median 1.7 KU/L, range 0.11–48) than in Tri a 19 positive ones (median 0.56 KU/L, range 0.11–49.2) (Mann-Whitney U test, $p = 0.0001$). A direct relationship was found between specific IgE levels for Tri a 14 and those for wheat (Spearman's, $\rho = 0.5303$; $p < 0.00001$). On the contrary, an inverse relationship was found among wheat IgE antibodies levels for Tri a 19 and those for wheat (Spearman's, $\rho = -0.3454$; $p = 0.0004$). Sensitization to Tri a 19 and Tri a 14 was independent as Tri a 14 positive patients were negative to Tri a 19 with a

significant inverse relation (Spearman's, $\rho = -0.7530$, $p < 0.00001$) (result obtained from the whole cohort of 124 selected patients). Interestingly, the 5 patients sensitized to both Tri a 14 and Tri a 19 had clinical features exclusively related to 1 or the other sensitization profile. A significant direct correlation was found between specific IgE levels for Tri a 14 and those for other cereals, i.e., barley, oats, rye, maize, and rice. In contrast, statistically significant inverse relations were found among Tri a 19-specific IgE levels and those for individual cereals, except for rye (for which a direct correlation was found) (shown in

Table 3. Correlation between Tri a 19 or Tri a 14 specific IgE levels with those for different foods, inhalants, and Pru p 3

Patients, N	Foods	Tri a 19		Tri a 14	
		p value	ρ	p value	ρ
102	Wheat	<0.0001	-0.345	<0.00001	0.530
77	Rice	<0.00001	-0.677	<0.00001	0.75
87	Maize	<0.00001	-0.760	<0.00001	0.878
59	Barley	<0.00001	-0.656	<0.00001	0.741
51	Oats	<0.00001	-0.642	<0.00001	0.802
55	Rye	0.505	0.092	<0.0093	0.348
79	Nut	<0.00001	-0.653	<0.00001	0.869
82	Peanut	<0.00001	-0.6913	<0.00001	0.7952
60	Hazelnut	<0.00001	-0.6576	<0.00001	0.7077
64	Almond	<0.00001	-0.5264	<0.00001	0.6074
60	Shrimp	<0.00001	0.5242	<0.00001	-0.4846
95	Pru p 3	<0.00001	-0.6783	<0.00001	0.8642

Patients, N	Inhalants	p value	ρ	p value	ρ
94	Grass	0.0001	-0.3815	0.0001	0.2553
110	Birch	<0.00001	-0.5137	0.0001	0.3683
80	<i>Dermatophagoides pt.</i>	0.3485	0.1060	0.5842	-0.0621

Table 3). This means that the higher the Tri a 19 IgE levels, the lower the IgE levels for other cereals. A significant inverse relation was observed between specific IgE levels for Tri a 19 and those for Pru p 3 (Spearman's, $\rho = -0.6703$, $p < 0.00001$). On the contrary, a direct correlation with high degree was documented between IgE levels for Tri a 14 and those for Pru p 3 (Spearman's, $\rho = 0.8633$, $p < 0.00001$). A significant direct relation was observed among specific IgE antibodies levels for Tri a 14 and specific IgE antibodies levels for nuts such as almond, walnut, hazelnut, and peanut (in all cases giving $p < 0.00001$) (Table 3). This can be traced back to the presence of LTP in all these allergenic sources. Specific IgE levels for Tri a 19 were instead significantly inversely correlated with specific IgE levels for all nuts aforementioned. Sensitization to shrimp was found in 30/79 (38%) patients among those sensitized to Tri a 14 and in 30/40 (75%) patients among those sensitized to Tri a 19. A statistically significant direct relationship was found among IgE antibody levels for Tri a 19 and those for shrimp (Spearman's, $\rho = 0.542$, $p < 0.00001$). On the other hand, an inverse relationship was found among IgE antibodies levels for Tri a 14 and those for shrimp (Spearman's, $\rho = -0.4846$, $p < 0.00001$).

Concerning inhalant allergens, we found a direct correlation between birch-specific IgE levels and Tri a 14

specific IgE antibodies, while an inverse correlation was found between specific IgE levels for Tri a 19 and those for birch (shown in Table 3). No other significant correlation was found.

Discussion

We compared the clinical characteristics of 2 populations of wheat allergic subjects sensitized to 2 different wheat allergens, namely Tri a 14 and Tri a 19. First of all, we found that the 2 sensitizations were independent, with a significant inverse relation in their specific IgE levels (Spearman's, $\rho = -0.7530$, $p < 0.00001$).

A possible, albeit indirect, confirmation of this comes from a recent European study that showed a higher frequency of wheat anaphylaxis among patients from central Europe, and in particular from Germany, where almost all patients were sensitized to Tri a 19, compared with that observed among patients from southern Europe [16]. Component-resolved diagnosis is not reported for the latter patients, but it is well acknowledged that in southern Europe sensitization to LTP in general is very frequent and it is therefore possible to assume that it was the cause in this group that exerted a preventive effect on Tri a 19 sensitization.

The independent sensitization to one of the 2 allergens in the present studied population seems to show a protective role of 1 sensitization over the other. The prevalence of atopy reached 67.1% among subjects sensitized to LTP compared with 32.5% among subjects sensitized to Tri a 19 (Fisher's exact test, $p < 0.0001$). Moreover, Tri a 19 positive subjects were significantly older than Tri a 14 sensitized patients (Fisher's exact test, $p < 0.001$), with a preponderance of male individuals. Male dominance also emerged in Kraft's work concerning omega-5-gliadin positive subjects.

The most striking result, however, concerns the risk of developing clinical symptoms as a function of the 2 sensitizations. In the case of subjects sensitized to Tri a 14, 44.3% (35/79) had never had symptoms induced by wheat consumption, and the specific IgE assay was requested as a complement to a diagnosis of LTP syndrome in subjects with sensitization to Pru p 3. If we sum patients with a negative challenge to these asymptomatic subjects, we can say that in 72.1% (57/79) of the cases of sensitization to wheat, LTP was asymptomatic. This result is in agreement with the 32% of Tri a 14 sensitized patients who reported symptoms with wheat described by San Bartolomé in a recent study [17]. This shows that, in a Pru p 3 allergic subject with LTP syndrome, the presence of Tri a 14 specific IgEs can be devoid of clinical meaning in most cases and the clinical reactivity should always be controlled by a food challenge to avoid a useless and dangerous wheat-free diet. In the group of patients sensitized to Tri a 19, a clear clinical history of multiple generalized reactions to wheat was present in 85% of patients, some of which were very severe after exertion and wheat consumption. Sensitization to Tri a 19, therefore, confers a significantly higher risk of having symptoms than sensitization to Tri a 14 (Fisher's exact test, $p < 0.0001$). Interestingly, despite a clearly positive history, all the patients who were Tri a 19 positive, who performed challenge plus exercise had not symptoms in agreement with the studies by Christensen, showing that only the highest amounts of gliadin can provoke the reaction [18]. This is useful as the ingestion of wheat far from exercise helps to overcome the disease. Regarding the severity of reactions, 55% (22/40) of the patients sensitized to omega-5-gliadin presented severe anaphylactic reactions compared with 19% (15/79) of the patients sensitized to LTP (Fisher's exact test, $p < 0.0005$). Also in Kraft's study, 66% of the subjects with omega-5-gliadin allergy had severe reactions [16].

A further important difference between the 2 groups was the dependence of reactions to wheat on the pres-

ence of cofactors, including, in particular, physical exertion, observed in 80% of Tri a 19 positive subjects compared to only 15.6% of those who were Tri a 14 positive (Fisher's exact test, $p < 0.0005$). An interesting finding was the association with alcohol consumption as this was observed as a cofactor of allergic reaction in 35% (14/40) of Tri a 19 positive subjects compared to 3.8% (3/79) of Tri a 14 sensitized subjects (Fisher's exact test, $p < 0.0005$). In this group, in general the history of alcohol consumption was negligible. The role of alcohol consumption or even alcohol abuse in facilitating WDEIA from omega-5-gliadin sensitization should therefore be assessed. The higher prevalence in the male population, in older subjects and absence of atopy, seems to lead to the suspicion that specific alterations induced on intestinal absorption by aggressive environmental factors including alcohol, may facilitate this clinical picture. Recently, a pathogenetic hypothesis of allergic diseases has been proposed, based on the loss of function of the epithelial barrier caused by aggressive agents that induce inflammation with translocation of the microbiota and triggering of a Th2 inflammatory reaction [19]. Alcohol could be one of these.

On the contrary, NSAIDs consumption as a trigger of the reaction did not differ between the 2 groups. However, both groups reported a very low use of NSAIDs and this can explain the difference with the study by San Bartolomé, where cofactors were present in 45% of wheat-related reactions among patients allergic to Tri a 14 [17]. The difference in the prevalence of cofactors compared to our study, however, is due to the fact that we wanted to assess how these affect the possibility of facilitating allergic symptoms in a population of subjects sensitized to Tri a 14.

Of great interest was the study of the immunological correlations of specific IgE for wheat and single allergens in the 2 populations, as only in the LTP positive subjects the IgEs for Tri a 14 was significantly correlated with that for wheat (Spearman's, $\rho = -0.530$, $p < 0.0001$), while in the Tri a 19 positive subjects an inverse correlation was even noted (Spearman's, $\rho = -0.354$, $p < 0.0001$). This is probably caused by the low presence of this protein in the allergen of the "in vitro" test. The same negative correlation occurred with IgEs for all other cereals except rye (shown in Table 2), whose sensitization was totally independent. Furthermore, the presence of omega-5-gliadin homologues in other cereals besides wheat is not known, although the clinical history of spelt-induced anaphylaxis among 2 of the Tri a 19 sensitized patients suggests the possible presence of this protein in this cereal too. On the

contrary, IgEs for Tri a 14 were significantly correlated with those for all other cereals. This is not surprising as LTP is known to be the major allergen in maize and rice with high homology to wheat. Sensitization to Tri a 14 was found to be directly related to sensitization to many other LTP related foods including peanut, walnut, hazelnut, and almond ($p < 0.0001$) as already described [20–22]. Conversely, the correlation between sensitization to Tri a 19 and shrimp, which in most subjects preceded that for wheat, was unexpected.

In recent decades, high-gluten wheat feed has been introduced as a protein substitute for crustacean farming, accounting for up to 35% of the feed as recommended by the Food and Agriculture Organization of the United Nations for shrimp aquaculture [23]. As the digestibility of this food by crustaceans is not fully known [24], it may remain undigested and could cause sensitization in susceptible individuals, creating new allergens shared with the shrimps or making shrimp sensitization easier.

In conclusion, the results of our study showed that sensitization to Tri a 14 excludes sensitization to Tri a 19, which is more frequently associated with severe symptoms, the absence of atopy, and predominantly affects older male individuals than those sensitized to Tri a 14. The latter, on the other hand, are mostly asymptomatic, so subjects with a Tri a 14 positive test should always undergo a food challenge.

Statement of Ethics

This study protocol was reviewed and approved by the Ethics Committee of ASST Grande Ospedale Metropolitano Niguarda (518-102018, Approved on October 26, 2018). Written informed consent has been obtained from participants to participate in the study.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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Author Contributions

All the authors have contributed equally to the design, data collection, realization, and writing of this manuscript.

Data Availability Statement

The data that support the findings of this study are openly available in Mendeley data at <http://doi.org/10.17632/ww3k4gfm-mj.1>.

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