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L. A. Gai[1] https://orcid.org/0000-0002-2934-7361
M. M. Kurtova[1] https://orcid.org/0000-0003-4060-619X
Ye. V. Tarasov[1] https://orcid.org/0000-0001-6809-5921
O. V. Kovaliuk[2] https://orcid.org/0000-0001-6789-7279
I. H. Koltsova[1] https://orcid.org/0000-0001-8784-7068

IMPACT OF ANTIBODIES TO CCD ON THE RESULTS OF SEROLOGICAL TESTING FOR FOOD ALLERGY

[1]Odesa National Medical University, Odesa, Ukraine
[2]Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine

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Background. Serological tests for food allergy can yield false positive results due to antibodies against cross-reactive carbohydrate determinants (CCD), potentially leading to overdiagnosis of IgE-mediated food allergy.

Objective – to assess the impact of anti-CCD antibodies on the results of serological testing for food allergy in patients from southern Ukraine.

Methods. A total of 1.210 patients with a suggestive or convincing history of food allergy underwent serological testing for specific IgE antibodies to various food allergens using two immunoblotting panels. One panel included a CCD marker. Statistical analysis compared the frequency and pattern of positive results in patients with and without anti-CCD antibodies.

Results. Antibodies to at least one allergen were detected in 46.0% of patients. Patients with anti-CCD antibodies had a higher count of positive reactions to CCD-containing allergens (mean: 7.30 vs. 0.85, p = <0.001*), while no such significant difference was observed for non-CCD allergens (mean: 1.15 vs. 1.90, p = 0.0256).

Conclusion. The presence of anti-CCD antibodies significantly increases the number of positive reactions to CCD-containing food allergens in serological tests. Multiple positive results for such allergens should prompt suspicion of anti-CCD antibodies and further evaluation to avoid overdiagnosis of food allergy.

Keywords: food allergy, cross-reactive carbohydrate determinants, CCD, IgE, Ukraine.

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Л. А. Гай[1], М. М. Куртова[1], Є. В. Тарасов[1], О. В. Ковалюк[2], І. Г. Кольцова[1]
ВПЛИВ АНТИТІЛ ДО CCD НА РЕЗУЛЬТАТИ СЕРОЛОГІЧНОГО ТЕСТУВАННЯ НА ХАРЧОВУ АЛЕРГІЮ
[1]Одеський національний медичний університет, Одеса, Україна
[2]Національний університет охорони здоров'я України імені П. Л. Шупика, Київ, Україна

Серед 1210 пацієнтів із підозрою або підтвердженим анамнезом харчової алергії у південному регіоні України у 46,0% виявлено антитіла хоча б до одного алергену з двох панелей. Вперше в Україні досліджено вплив антитіл до перехресно-реактивних вуглеводних детермінант (CCD) на результати серологічного тестування: у носіїв анти-CCD антитіл кількість позитивних реакцій на CCD-вмісні алергени була суттєво вищою (у середньому 7,30 проти 0,85; p<0,001), тоді як для алергенів без CCD такої різниці не виявлено. Отже, множинні позитивні результати на CCD-вмісні харчові алергени під час тестування екстрактами повинні викликати підозру на наявність анти-CCD антитіл і є підставою для додаткового обстеження з метою уникнення надмірної діагностики IgE-опосередкованої харчової алергії.

Ключові слова: харчова алергія, перехресно-реактивні вуглеводні детермінанти, CCD, IgE, Україна.

Introduction

Current diagnostic approaches to patients with suspected food allergy include history and physical examination, skin prick testing (SPT), in vitro testing and food challenges [1; 2]. In patients with convincing or suggestive history, the initial testing includes SPT or in vitro tests, which are mainly carried out by serological methods using enzyme immunoassay or immunoblotting. The choice of testing depends on patient clinician preferences, tests availability, and limitations. Although immunoassays are more costly than SPT, they are not affected by antihistamines and other medications, or skin conditions, and can be safely used in patients with severe anaphylaxis. Unfortunately, immunoassays may demonstrate false positive results due to the antibodies to cross-reactive carbohydrate determinants (CCD), which are protein-linked carbohydrate structures common in food allergens of plant origin [3]. Being tested for specific IgE (sIgE) antibodies by serological assays, patients with antibodies to CCDs can show a large number of positive reactions, called multi-reactions. This may lead to confusing results and overdiagnosis of IgE-me-

diated food allergy. Previously, we have shown the effect of anti-CCD antibodies on immunoassay results in patients with respiratory allergy [4]. Here we explore the possible role of anti-CCD antibodies in initial in vitro testing for suspected IgE-mediated food allergy.

The purpose of the study is to explore the possible role of anti-CCD antibodies in initial *in vitro* testing for suspected IgE-mediated food allergy.

Materials and methods

1210 patients of the Southern region of Ukraine (Odesa and Mykolayiv regions) with suggestive or convincing history of food allergy administered for initial serological testing by clinicians. Patients were tested for sIgE using two distinctimmunoblotting panels with different sets of crude allergen extracts produced by Mediwiss (Germany): 238 patients were tested using Mediwiss Food LV30 (Panel B), that contains CCDx marker, 972 with Mediwiss Food A30 (Panel A) without CCDx marker. The following allergens were tested: Mediwiss Food LV30 – CCDx, Staphyl. mix (mstaph), codfish (f3), salmon (f41), beef (f27), pork (f26), chicken (f83), egg white (f1), egg yolk (f75), milk (f2), α-lactalbumin (f76), β-lactoglobulin (f77), caseine (f78), wheat flour (f4), rye flour (f5), oat flour (f7), maize flour (f8), peanut (f13), coconut (f36), tomato (f25), potato (f35), bell pepper (f218), buckwheat (f11), carrot (f31), celery (f85), apple (f49), orange (f33), banana (f92), soy bean (f14); Mediwiss Food A30 – milk (f2), caseine (f78), cheese mix (fx400), egg white (f1), egg yolk (f75), beef (f27), fish mix (fx3), shrimp (f24), cacao (f93), wheat flour (f4), barley flour (f6), rice flour (f9), maize flour (f8), soy bean (f14), sesame seed (f10), carrot (f31), potato (f35), tomato (f25), celery (f85), banana (f92), citrus mix (fx10), pepper (f263), strawberry (f44), kiwifruit (f84), onion (f48), peanut (f13), hazelnut (f17), walnut (f256), almond (f20), pistachio nut (f203). CCDx marker included in the Mediwiss Food LV30 panel contains mixture of the CCD-containing molecules – bromelain, horseradish peroxidase and ascorbate oxidase, usually used for detection of anti-CCD antibodies.

Reaction was assumed as positive if concentration of IgE was equal or more than 0.35 IU/ml (1 class according to RAST classification).

This study is in compliance with the principles of the World Medical Association Code of Ethics and was approved by the ethics committee of Odessa National Medical University (Protocol No. 9 – 04.11.2019). Written informed consent was obtained from each patient or patient's parents in case if the patient has not reached 18 years old.

All data processing and statistical analyses were performed using R (version 4.0 or later) and RStudio. The following R packages were primarily used: tidyverse for data manipulation and visualization (including ggplot2 for plots and dplyr for data wrangling), readxl for importing Excel data, DescTools for calculating Wilson binomial confidence intervals (BinomCI), knitr for generating HTML tables (kable), and MASS for Negative Binomial Generalized Linear Models (glm.nb). Standard R functions from the stats package were used for Wilcoxon Rank-Sum tests (wilcox.test) and Poisson Generalized Linear Models (glm with family = poisson). Frequencies are presented as percentages with 95% Wilson CIs. Comparisons of counts between groups were performed using the non-parametric Mann-Whitney U test (Wilcoxon rank-sum test). For count data, Poisson and Negative Binomial GLMs were also employed to model the relationship between group status and allergen counts, providing an alternative statistical approach. For inter-panel comparisons of individual allergen frequencies (binary data: positive/negative), the Mann-Whitney U test was used. A p-value < 0.01 was considered statistically significant for all tests.

Results and their discussion

Among patients with suggestive or convincing history of food allergy in the Southern region of Ukraine (N=1210), 46.0% [95% CI: 43.2, 48.8] had antibodies to at least one allergen across the two panels. Gender and age characteristics of seropositive patients were as follows: For Panel A: the panel initially included N = 402 seropositive participants, the mean age was M = 22.03 years (SD = 18.48), sex distribution: Female: n = 208 (51.7%), Male: n = 194 (48.3%); For Panel B: the panel initially included N = 154 seropositive participants, the mean age was M = 20.81 years (SD = 17.82), sex distribution: Female: n = 82 (53.2%), Male: n = 72 (46.8%).

Both panels showed similar total positive allergen distribution (Figure 1).

Inter-Panel Comparison of composition of positive allergen count were analyzed (Figure 2). It was shown that there were no significant difference in Overall Total Positive Allergens Count (p = 0.54840), Overall CCD-Containing Allergens Count (p = 0.43130) and Overall Non-CCD Allergen Count (p = 0.22300).

Due to different allergens included in used panels the results were analyzed separately (Table 1). In Panel A, the most commonly detected antibodies were to Egg white (f1) (41.3%; 95% CI: [36.6, 46.2]) followed by Milk (f2) (40.3%; 95% CI: [35.6, 45.2]). In Panel B, the structure of seropositivity showed antibodies to α-lactalbumin (f76) (40.3%; 95% CI: [32.8, 48.2]) followed by Milk (f2) (39.6%; 95% CI: [32.2, 47.5]) as most common.

The highest detection rates for CCD-containing food allergens were for Bell pepper (f218) (27.9%; 95% CI: [21.4, 33.5]) and Wheat flour (f4) (25.6%; 95% CI: [21.6, 30.1]) (Panel A)/ (20.1%; 95% CI: [14.6, 27.2]) (Panel B).

sIgE detection rate to CCD was 13.0%; 95% CI: [10.29, 15.71], which was comparable to anti-CCD antibodies detection rate in our study of patients with suspected respiratory allergy – 17.8%; 95% CI: [16.09, 19.51] [5]), as well as other studies [3; 4].

It should be noted that for some non-CCD allergens (Table 1, marked with asterisk) surprisingly significant difference in detection rate in two panels of the same manufacturer was observed. The nature of this phenomenon should be investigated separately.

Earlier we showed that the presence of anti-CCD antibodies significantly changed results of immunoassays for respiratory allergy testing by increasing detection rate of antibodies to plant and insect allergens, and number of positive markers on a panel [5; 6]. Therefore, we assumed

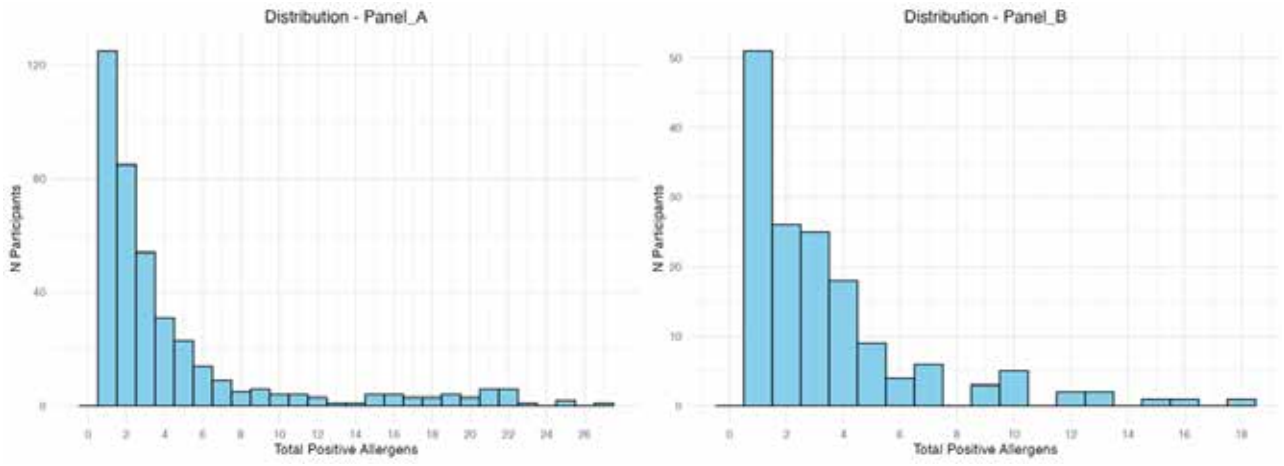

Fig. 1. Distribution of Total Positive Allergens in Panels A and B

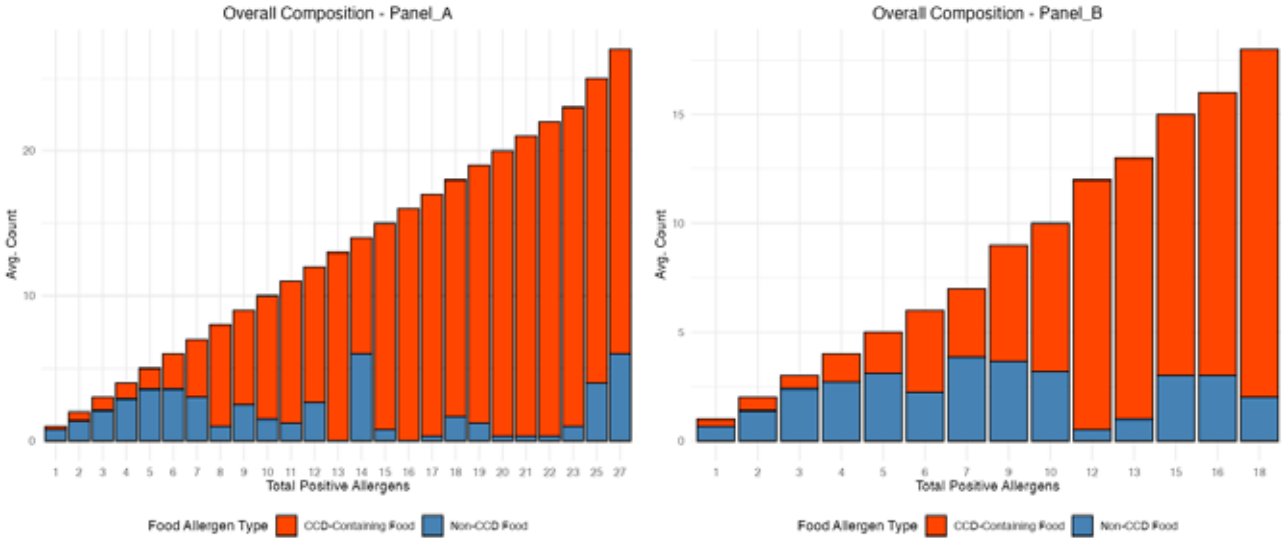

Fig. 2. Overall Composition of Positive Allergens

Table 1

Combined Allergen Frequency Table (Panel A vs Panel B)

Allergen	Panel A		Panel B		p-value (A vs B)
	Freq (%)	95% CI	Freq (%)	95% CI	
1	2	3	4	5	6
CCD-containing Allergens					
Cacao (f93)	2.7%	[1.5, 4.8]	–	–	–
Wheat flour (f4)	25.6%	[21.6, 30.1]	20.1%	[14.6, 27.2]	0.176
Barley flour (f6)	11.4%	[8.7, 14.9]	–	–	–
Rice flour (f9)	14.4%	[11.3, 18.2]	–	–	–
Maize flour (f8)	21.1%	[17.4, 25.4]	14.3%	[9.6, 20.7]	0.0667
Soy bean (f14)	7.5%	[5.3, 10.5]	3.9%	[1.8, 8.2]	0.127
Sesame seed (f10)	14.2%	[11.1, 17.9]	–	–	–
Carrot (f31)	13.4%	[10.4, 17.1]	7.8%	[4.5, 13.1]	0.0661
Potato (f35)	13.2%	[10.2, 16.8]	7.1%	[4.0, 12.3]	0.046
Tomato (f25)	13.2%	[10.2, 16.8]	7.8%	[4.5, 13.1]	0.077
Celery (f85)	19.4%	[15.8, 23.6]	11.0%	[7.0, 17.0]	0.0192
Banana (f92)	7.7%	[5.5, 10.7]	3.9%	[1.8, 8.2]	0.107
Citrus mix (fx10)	15.7%	[12.4, 19.5]	–	–	–
Pepper (f263)	8.7%	[6.3, 11.9]	–	–	–
Strawberry (f44)	5.5%	[3.6, 8.1]	–	–	–

Continuation of Table 1

1	2	3	4	5	6
CCD-containing Allergens					
Kiwifruit (f84)	12.0%	[9.1, 15.5]	–	–	–
Onion (f48)	10.7%	[8.0, 14.1]	–	–	–
Peanut (f13)	17.4%	[14.0, 21.4]	12.3%	[8.0, 18.5]	0.145
Hazelnut (f17)	22.9%	[19.0, 27.2]	–	–	–
Walnut (f256)	12.9%	[10.0, 16.6]	–	–	–
Almond (f20)	5.7%	[3.8, 8.4]	–	–	–
Pistachio nut (f203)	11.7%	[8.9, 15.2]	–	–	–
Rye flour (f5)	–	–	15.6%	[10.7, 22.1]	–
Oat flour (f7)	–	–	2.6%	[1.0, 6.5]	–
Coconut (f36)	–	–	5.2%	[2.7, 9.9]	–
Bell pepper (f218)	–	–	27.9%	[21.4, 35.5]	–
Buckwheat (f11)	–	–	15.6%	[10.7, 22.1]	–
Apple (f49)	–	–	4.5%	[2.2, 9.1]	–
Orange (f33)	–	–	9.1%	[5.5, 14.7]	–
Non-CCD Allergens					
Milk (f2)	40.3%	[35.6, 45.2]	39.6%	[32.2, 47.5]	0.883
Caseine (f78)	9.2%	[6.8, 12.4]	17.5%	[12.3, 24.3]	**0.00595***
Cheese mix (fx400)	28.4%	[24.2, 33.0]	–	–	–
Egg white (f1)	41.3%	[36.6, 46.2]	25.3%	[19.1, 32.7]	**<0.001***
Egg yolk (f75)	21.9%	[18.1, 26.2]	9.7%	[6.0, 15.4]	**<0.001***
Beef (f27)	13.7%	[10.7, 17.4]	4.5%	[2.2, 9.1]	**0.00222***
Fish mix (fx3)	3.7%	[2.3, 6.1]	–	–	–
Shrimp (f24)	4.0%	[2.5, 6.4]	–	–	–
Staphyl. mix (mstaph)	–	–	19.5%	[14.0, 26.4]	–
Codfish (f3)	–	–	2.6%	[1.0, 6.5]	–
Salmon (f41)	–	–	1.9%	[0.7, 5.6]	–
Pork (f26)	–	–	5.2%	[2.7, 9.9]	–
Chicken (f83)	–	–	1.9%	[0.7, 5.6]	–
α-lactalbumin (f76)	–	–	40.3%	[32.8, 48.2]	–
β-lactoglobulin (f77)	–	–	11.7%	[7.5, 17.7]	–

** Statistically significant ($p < 0.01$). '–' indicates allergen absent in panel or no valid observations.*

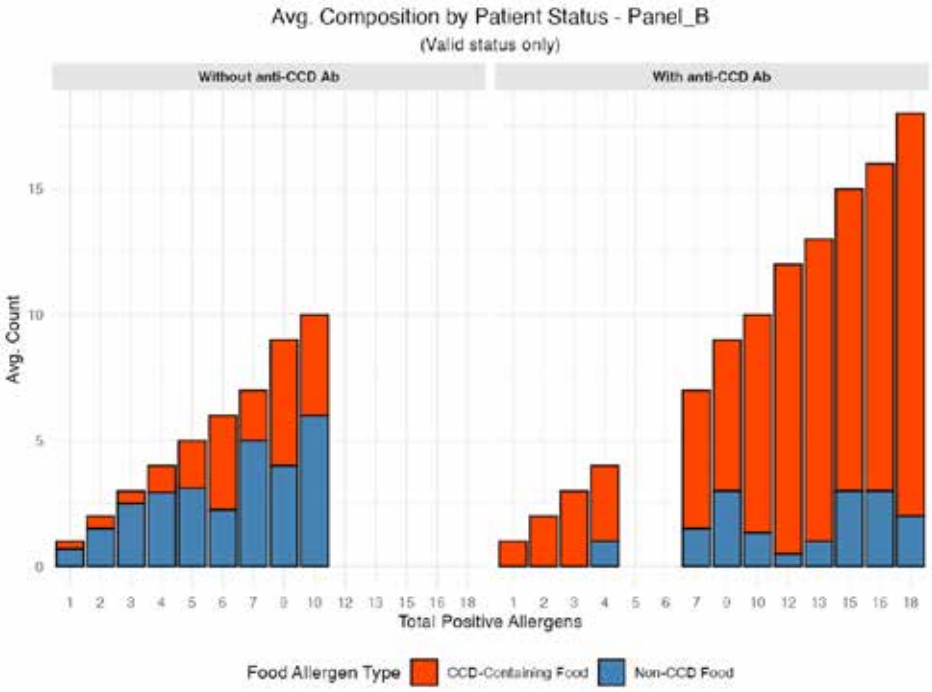

Fig. 3. Intra-panel analysis of Panel B. Faceted view by patient CCD antibody status

that the same pattern would be maintained for food allergens if crude allergen extracts were used.

To test this hypothesis, we performed intra-panel comparison for panel B since it had CCDx marker allowing us to compare results of patients without anti-CCD antibodies and patients with anti-CCD antibodies.

Analysis of the number of positive markers on one immunoblot in Panel B showed that the proportion of CCD-containing food allergens increased significantly (p = <0.001*) in patients with anti-CCD antibodies compared to those without. Specifically, patients with anti-CCD antibodies had a higher count of positive reactions to CCD-containing allergens (mean: 7.30 vs. 0.85, p = <0.001*), while no such significant difference was observed for non-CCD allergens (mean: 1.15 vs. 1.90, p = 0.0256) (Figure 3).

Thus, multiple positive reactions to CCD-containing food allergens using tests with extract allergens should raise a suspicion for the presence of anti-CCD antibodies and warrant further investigation to avoid overdiagnosis of specific IgE-mediated food allergy.

Conclusions

Among patients in southern Ukraine with a suggestive or confirmed history of food allergy, 46.0% 95% CI: 43.2, 48.8 tested positive for antibodies to at least one allergen from the two panels analyzed. In Panel A, antibodies were most frequently detected against egg white (f1) (41.3% 95% CI: 36.6, 46.2), followed by milk (f2) (40.3% 95% CI: 35.6, 45.2). In Panel B, the highest seropositivity was observed for lactalbumin (f76) (40.3% 95% CI: 32.8, 48.2), with milk (f2) as the next most common (39.6% 95% CI: 32.2, 47.5). The effect of antibodies to cross-reactive carbohydrate determinants (CCDs) on food allergy seropositivity was evaluated for the first time in Ukraine using Panel B, which included a CCDx marker.

For the first time in Ukraine, the influence of antibodies to cross-reactive carbohydrate determinants (CCDs) on food allergy seropositivity was examined using Panel B, which includes a CCDx marker. Analysis revealed a significantly higher number of positive results for CCD-containing food allergens in patients with anti-CCD antibodies compared to those without (mean: 7.30 vs. 0.85). There was no significant difference for non-CCD allergens (mean: 1.15 vs. 1.90). Therefore, when multiple positive reactions to CCD-containing food allergens are detected using extract-based tests, the presence of anti-CCD antibodies should be suspected, and further evaluation is recommended to prevent overdiagnosis of specific IgE-mediated food allergy.

Conflict of Interest. The authors declare that there is no conflict of interest and no financial interest in the preparation of this article.

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Електронна адреса для листування gruzevskiy@ua.fm