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Correlation between Component-resolved Diagnostics (CRD) and Clinical Symptoms in Allergic Children: A One-year Study at the Children's Medical Center (April 2023–March 2024)

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ABSTRACT

Component- Resolved Diagnosis (CRD) is an effective tool in allergy diagnosis, that detects specific IgE to allergenic molecules. The ALEX (Allergy Explorer) test, commercially available since 2019, measures specific IgE to allergenic extracts and components associated with inhalant, food, animal, latex, and insect allergens. CRD results should be interpreted based on the patient's clinical history.

Since Children's Medical Center Hospital is one of the largest referral centers for allergic patients, we evaluated the results of the ALEX2 test in patients referred to this center and compared them with the patients' clinical symptoms.

Clinical symptoms were concordant with positive CRD (ALEX2) test in 76.7% of cases. The overall agreement between positive allergen components and clinical symptoms was 58%.

These findings indicate that the ALEX2 test can improve diagnostic accuracy in allergic patients; however, positive test results should be interpreted in the context of the patient's clinical history.

Keywords: Allergy; Clinical symptoms; Component resolved diagnosis

INTRODUCTION

Allergy is an increasingly common condition among populations. Allergic reactions are commonly associated with a variety of foods, pollens, medications, and other sources. The molecules in these substances that cause abnormal allergic reactions are known as allergens. Estimates of allergy prevalence vary; in developed countries and across the lifespan, the overall prevalence of food allergy is estimated to be 4% to 7% among children and 3% to 6% among adults.[1,2] In addition, respiratory allergies affect approximately 10% to 25% of the world's population and have been steadily increasing over the past three decades.[3] Therefore, obtaining a focused clinical history for allergies is essential to identify potential allergic triggers and guide testing.[4]

The clinical history is key to an accurate diagnosis; in cases with a clear history of typical IgE-mediated symptoms after ingestion of a specific food, the diagnosis can be relatively straightforward. However, in

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the absence of a clear history, when an allergic reaction is unclear, or with indiscriminate consumption of a suspected allergenic food, the diagnosis will be much more challenging. The diagnosis of IgE-mediated food allergy requires evidence of IgE sensitization by skin prick testing (SPT) or serum IgE. The diagnosis is often made by a combination of history with allergen-specific IgE testing.[5]

Laboratory approaches to diagnosing allergic disease revolve around the detection of specific immunoglobulin E (sIgE) directed against potential allergen sources based on the patient's history. The allergens used in these tests are complex because extracts from a single allergen source contain many different components. Advances in laboratory techniques have led to the development of tests that detect sIgE against molecular allergen components. The general term for this concept is component resolved diagnosis (CRD).[6] The ability to distinguish between sIgE against individual components allows clinicians to better differentiate true allergen sensitization from cross-reactivity. Recent reviews of the use of CRD in allergy testing across the UK and Europe indicate that the use of CRD is increasingly incorporated into routine diagnostics.[4] Component-resolved diagnosis is being rapidly incorporated into clinical use, and sophisticated diagnostic tests that indicate severity and prognosis are on the horizon.[7]

CRD involves the detection of specific IgE (sIgE) levels to allergen molecules or epitopes of those allergens.[8] Certain CRD components have demonstrated allergenic potential with higher specificity and lower sensitivity than conventional first-line tests.[9]

CRD technologies are commercialized by Thermo Fisher, Hycor, Euroline, and Siemens, MADx.[10]
ALEX2 is the first multiplex assay on the market that concurrently assesses allergen extracts and components.[11] It is a novel tool for characterizing IgE profiles, in which IgE assays should be performed on many allergens and their components.[12] in addition ALEX2 Allergy Xplorer is the same equivalent of MacroArray Diagnostics.

ALEX is a relatively new multiplex allergy test that analyzes more than 120 allergen extracts and 170 molecular components from inhalant, food, animal, latex, and insect allergen sources. This array contains 295 allergens reagents. ALEX² is an immunoassay test based on enzyme-linked immunosorbent assay (ELISA). The presence of a larger number of allergens in the CRD could help clinicians improve recommendations. However, CRD also yields a greater number of positive sensitizations of uncertain clinical relevance, which may increase clinical complexity.[13]

At present, the application and routine use of CRD are in their infancy and requires immunologists, laboratories, and clinicians to evaluate new evidence for their use in the absence of standardized protocols. Increased use of CRD by clinicians and allergists is needed to provide useful clinical and laboratory information in terms of sensitivity, specificity, and risk assessment. However, there is still a long way to go before CRD is routinely used and interpreted with confidence.[4] CRD is an effective tool for allergy diagnosis that offers the potential to identify specific phenotypes and create risk profiles tailored to each patient.[4] The diagnostic accuracy of identified components varies across studies, and the diagnostic value and clinical utility of CRD remain unclear.[14,15] Multiplex analysis of molecular components is considered a third-level diagnostic tool after the SPT and singleplex sIgE testing for total allergen extracts. Multiplex instruments help clinicians accurately diagnose allergies by distinguishing between clinically relevant cases. The purpose of this study was to investigate the correlation between CRD test results and clinical symptoms of patients referred to the Allergy Clinic of the Children's Medical Center during 1 year (April 2023 to March 2024).

MATERIALS AND METHODS

Given that this is a cross-sectional analytical study, patients with allergy symptoms referred to the Allergy Clinic of the Children's Medical Center and were enrolled after confirmation and diagnosis by an allergy and ng parental or patient consent. Respiratory allergies including asthma, allergic rhinitis, food allergies, anaphylaxis, skin allergies (atopic dermatitis and urticaria), and gastrointestinal allergies were examined. All patients then underwent CRD testing with the ALEX2 platform. This test is produced by MacroArray Diagnostics GmbH from Austria (Vienna), which was based on nano-bead technology. The sampling method involved collecting a blood sample with a volume of 1 cc. The relationship between allergic sensitization and clinical symptoms was determined based on the patient's history According to the test results, appropriate treatment measures were initiated. The primary tool for

data collection in this study was a questionnaire. Information was extracted in the form of a questionnaire prepared from patients referring to the clinic. Interviews with the patient's family and review of the patient's file documents were additional data-collection methods. The questionnaire was developed based on a pilot sample of patients. All patients referred to the Allergy Clinic of the Children's Medical Center who met eligibility criteria were included in the study. After obtaining complete information and criteria in the file or available information and parental permission, all eligible patients were enrolled. The sampling method was census; the sample comprised all eligible patients seen between April 2023 and March 2024 (1 year).

Statistical Analysis

After collecting the required information, the data were analyzed using SPSS statistical software version 27 (IBM Corporation, Armonk, NY, USA). To describe quantitative data, the mean and standard deviation (SD) were used if the data distribution was normal. If it was not normal, the median and interquartile range were used. For qualitative data, frequency and percentage were reported. The χ^2 test was used to analyze the data. In addition to analyzing the results of the χ^2 test and percentages, the Kappa coefficient was calculated. The significance level was considered to be $p<0.05$. We also used a correspondence table to match clinical symptoms and test results.

RESULTS

A total of 120 ALEX2 tests with positive results (who all patients have positive ALEX2 test) were included; positive test results were compared with patients' clinical symptoms. Sixty-six cases (55%) were male and 54 cases (45%) were female. The mean (SD) age of allergic patients with a positive test was approximately 8 years (7.7). In terms of history and clinical symptoms, 47 patients had allergic rhinitis, 44 cases had asthma, 36 cases had anaphylaxis, 40 cases had a history of skin allergy and eczema, 35 had urticaria, and 17 had urticaria and eczema concurrently. Fifty-seven cases had a history and clinical symptoms of food allergy, and 10 had gastrointestinal allergy. Twenty-two patients had wheat allergy, 15 had egg allergy, 10 had nut allergy, 5 had fruit allergy, 4 had milk allergy, 1 had simultaneous allergy to eggs and potatoes, and 3 had allergies to more than two foods. Food anaphylaxis included wheat, milk, eggs, nuts, and fruits, and two cases were anaphylaxis due to venom. Among 47 patients with allergic rhinitis, 40 (85%) had concordant test and clinical results, and 7 (15%) had discordant results. Statistically, a kappa coefficient of 0.874 indicates very good agreement between laboratory and clinical results for allergic rhinitis. Of 44 patients with asthma and a positive CRD test, 39 (88.6%) had concordant test and clinical results; 5 (11.4%) were discordant. A significant association ($p<0.001$) was found between ALEX2 laboratory results and clinical diagnosis in asthma patients. The kappa coefficient was 0.908, indicating very good agreement between laboratory and clinical results for asthma. Of the 36 patients with symptoms of anaphylaxis, 28 cases had concordant laboratory and clinical findings (28/36, 77.8%); 8/36 (22.2%) were discordant. The results indicate a significant relationship between laboratory and clinical results in anaphylactic patients, and the kappa coefficient was 0.83, indicating high agreement between clinical and laboratory results. In patients with atopic dermatitis and eczema, 37 of 40 patients had concordant laboratory and clinical results (92.5%); 3/40 (7.5%) were discordant; also, median result for age of patient is 7. The kappa coefficient was 0.943, indicating a strong agreement between laboratory and clinical results. In patients with clinical symptoms of urticaria, 23 of 35 patients (65.7%) had concordant laboratory and clinical results, and 12/35 (34.3%) were discordant. The kappa coefficient was 0.731, indicating a relative agreement between laboratory and clinical results. This suggests relatively good accuracy of the test for urticaria. Overall, of 57 patients with a history of food allergy and positive laboratory results, 47 (82.5%) had concordant clinical and laboratory findings, and 10 (17.5%) were discordant. The kappa coefficient was 0.832, indicating high agreement and appropriate accuracy of the laboratory method for food allergy. The findings indicated a significant association between laboratory and clinical results in food allergy. Of 10 patients with gastrointestinal (GI) allergic symptoms (including eosinophilic esophagitis and allergic colitis) and positive CRD results, 7 (70%) had concordant laboratory and clinical findings, and 3 (30%) were discordant. The kappa coefficient was 0.811 for GI Allergy (Table 1).

Table 1. Agreement Between Clinical presentation and ALEX test findings

Types	Total patients	Matched percent	Kappa	Chi-square	*P*
Allergic rhinitis	47	85.1%	0.874	93.191	0.000
Asthma	44	88.6%	0.908	99.798	0.000
Anaphylaxis	36	77.8%	0.831	85.217	0.000
Skin/eczema	40	92.5%	0.943	106.988	0.000
Urticaria	35	65.7%	0.731	69.102	0.000
Food allergy	57	82.5%	0.832	85.393	0.000
GI allergy	10	70%	0.811	81.770	0.000

Overall, results show that 92 (76.7%) had concordant clinical and laboratory findings and 28 (23.3%) had discordant findings. In a component-level analysis, positive allergen components were evaluated for association with patients' clinical symptoms. Across the 120 positive ALEX2 tests, 1,532 positive molecular components were recorded; 891 (58.2%) were concordant with patients' clinical symptoms, while 641 (41.8%) were not. For respiratory allergies, the proportion of positive components concordant with clinical history was 60% for asthma and 59% for allergic rhinitis; the corresponding discordant proportions were 40% and 41%, respectively.

Among patients with food allergies, 60% of the identified allergen components demonstrated a clinically relevant association, whereas 40% represented positive laboratory findings without corresponding clinical symptoms. In cases of anaphylaxis, 64% of the allergen components detected via the ALEX2 multiplex allergy test were consistent with documented clinical manifestations, while 36% were positive laboratory results lacking clinical correlation. For patients presenting with cutaneous allergic conditions-including eczema, atopic dermatitis, and urticaria-51% of the positive allergen components were supported by clinical history, whereas 49% were laboratory findings not associated with observable symptoms (Table 2).

Among patients with respiratory allergies, the most frequently identified aeroallergens were:

Weed pollen, particularly Russian thistle, with molecular components *Sal k* and *Sal k 1*, both associated with pectin methyl esterase activity.

Grass pollen, including: Timothy grass, with the molecular component Phl p 1. Bermuda grass, with the molecular component Cyn d.

Tree pollen, with Cypress (Cupressus arizonica) being the most prevalent, identified by the molecular component Cup a 1.

Table 2. Correspondence between positive allergen components in the ALEX2 test and clinical symptoms by type of allergy

Type of allergy	Agreement	Kappa
Allergic rhinitis	59%	0.649
Asthma	60%	0.658
Food allergies	60%	0.658
Anaphylaxis	64%	0.692
Skin allergy	51%	0.582
Total	58%	0.640

In pediatric patients with food allergies, the most common allergens were Wheat, with molecular components Tri a aAT1, Tri a 14, and Tri a 19. Egg, with components from egg white including Gal d 1 (ovomucoid), Gal d 2 (ovalbumin), and Gal d white, which showed the strongest association with clinical symptoms. Cow's milk, with molecular components Bos d 4 (α-lactalbumin), Bos d 5 (β-lactoglobulin), and Bos d 8 (casein), all of which were frequently linked to clinical manifestations (Table 3).

Table 3. Most common inhalant allergens and food allergens

Molecule component	Function	Allergen	Number
	Weed pollen		
Sal k		Russian thistle	22
Sal k 1	Pectin methylesterase	Russian thistle	33
Ama r		Common pigweed	22
	Grass pollen		
Phl p 1	Beta-Expansins	Timothy grass	18
Cyn d	Beta-Expansins	Bermuda grass	15
	Tree pollen		
Cup a 1	Pectate lyase	Cypress	15
	Milk		
Bos d 4	α-Lactalbumin	Cow milk	6
Bos d 5	β-Lactoglobulin	Cow milk	8
Bos d 8	Casein	Cow milk	5
	Egg		
Gal d white		Egg white	17
Gal d yolk			5
Gal d 1	Ovomucoid		15
Gal d 2	Ovalbumin		16
Gal d 3	Ovotransferrin		7
Gal d 4	Lysozyme		6
Gal d 5	Serum Alb		5
	Wheat		
Tri a aA-T1	Alpha-amylase trypsin inhibitor		22
Tri a 14	nsLTP		22
Tri a 19	Omega 5- Gliadin		17

DISCUSSION

Out of 120 CRD (ALEX2) test results from patients presenting with allergic symptoms, 76.7% (n=92) demonstrated concordance between laboratory findings and clinical manifestations. Across the cohort, a total of 1,532 positive allergen components were identified, of which 891 (58%) were clinically relevant, while 641 (42%) lacked association with reported symptoms. Among patients with respiratory allergies, including allergic rhinitis and asthma, the correspondence between positive ALEX2 results and clinical symptoms was notably high-85% and 88.6%, respectively-underscoring the test's reliability in diagnosing respiratory allergic conditions. These findings align with those reported by González-Mancebo et al (2017), who compared CRD with SPT in patients sensitized to pollen. Their study found a higher detection rate with CRD, supporting its utility in allergy diagnostics. Moreover, CRD was shown to be a critical tool for guiding immunotherapy decisions and monitoring allergic disease progression. The consistency between CRD profiles and clinical sensitivity observed in their study parallels the results of the present investigation, further validating the role of CRD in comprehensive allergy assessment. In a study conducted by Eiringhaus et al (2019) they reported that CRD can provide more information about the patient's sensitivity profile, response to treatment, and diagnosis of allergy largely depends on the clinical history, physical examination, and IgE testing.[16] The results of our center's study also showed that the CRD test in patients with allergic rhinitis and asthma has a good correlation with the patients' symptoms. Therefore, this test can be used to identify inhaled allergens in these patients. In patients with anaphylaxis, 77.8% of positive laboratory results agreed with the patients' clinical symptoms, and the statistical results indicate a high agreement of the CRD test in these patients.

In the study by Luengo et al (2014) was shown that one indication for CRD testing was anaphylaxis. The results of the present study in anaphylaxis patients are consistent with previous studies.[14]

In patients with atopic dermatitis, 92.5% agreement between clinical and laboratory results was reported in our center's study, indicating a significant relationship.

A study conducted by Čelakovská et al (2022) examined allergic sensitization using the Alex2 test in 100 patients with atopic dermatitis and evaluated clinical reactions to fish and shrimp.[17] The results of this study showed a significant relationship between fish molecular components on the Alex2 test and the patients' clinical history. However, a significant relationship between the results of shrimp-specific IgE and the patients' clinical history was not confirmed.

In patients with food allergy and positive laboratory results, 82.5% agreement between clinical and laboratory findings was reported. The statistical results indicate that the Alex2 test has good accuracy for diagnosing food allergy.

In a systematic review by Flores et al (2018), they reported that CRD has high specificity for cow's milk, egg, peanut, hazelnut and shrimp. This method is a promising tool for diagnosing food allergy. In our present study, weed pollen (Russian thistle with molecular component *Sal k 1*) and tree pollen (cypress with component *Cup a 1*) were reported to be the most common allergens among patients with respiratory allergy.[9]

In the study by Ricci et al (2019) CRD can be useful in the diagnosis and management of wheat allergy patients. In our study, there were 22 cases of wheat anaphylaxis, and all patients had positive components *Tri a*, *Tri a aA-T1*.[18]

In contrast, in the study by González-Mancebo et al (2017), which profiled allergic sensitization using CRD and SPT in patients with walnut allergy, the most common sensitizing pollens were reported to be Olea and Grass.[19]

In children with food allergy, the most common allergens were wheat, eggs, and milk, respectively.

In the study by Foong et al conducted (2021), *Gal d 1* and *Gal d 2* were the main egg allergens.[5] In our study, *Gal d 1* and *Gal d 2* were most associated with clinical symptoms. In children with cow's milk allergy, *Bos d 4* (α-lactalbumin), *Bos d 5* (β-lactoglobulin), and *Bos d 8* (casein) were most closely associated with clinical symptoms. Additionally, the main cow's milk allergens were casein (*Bosd8*), β-lactalbumin (*Bos d 5*) and *Bos d 8* was shown to be a marker of persistent cow's milk allergy.

In the study by Li et al conducted in 2018, *Bos d 12* showed the best performance in diagnosing cow's milk allergy; *Bos d 12* is not measured in the test used by our center.[20]

In a study of 91 hazelnut allergy cases in Japan, Inoue et al (2020) showed that low sIgE levels for *Cor a 1* could improve the diagnostic accuracy for identifying hazelnut-sensitive Japanese children, and *Cor a 14* was

an effective predictor of hazelnut allergy.[21] This study demonstrated that CRD improved diagnostic accuracy for hazelnut allergy in Japanese children. In our study, due to the small number of patients with hazelnut allergy, results could not be examined or compared. Overall, 92 of 120 positive tests (76.7%) were accompanied by clinical symptoms in the patients; therefore, the ALEX2 test can substantially aid in the diagnosis of allergic diseases. Considering positive components, 58% concordance with patients' clinical symptoms was observed; thus, test results and identified components should be interpreted in the context of the patients' history and clinical examination. Further, patients should not be advised to avoid allergens solely on the basis of a positive test result. our findings indicate that the ALEX2 test can improve diagnostic accuracy in allergic patients, but positive results must be interpreted together with clinical history.

Some limitations of the study include the following: failure to compare the CRD components test and SPT simultaneously to assess their separate sensitivity and specificity, and failure to assess the economic aspects of CRD compared with other commonly used allergy tests.

Considering that this study was conducted in a pediatric medical center, we recommend that the same study be conducted in several other centers with broader geographical distribution and larger patient cohorts. These topics can be addressed in future research.

STATEMENT OF ETHICS

The Ethics Committee of Tehran University of Medical Sciences approved this study (IR.TUMS.CHMC.REC.1402.173).

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

All the data are available in the article.

AI ASSISTANCE DISCLOSURE

Not applicable.

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