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Development and Validation of An Integrated Molecular Allergy Diagnostic Algorithm for Precision Evaluation of Children with Atopic Dermatitis

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Abstract

Background

Atopic dermatitis (AD) is one of the most prevalent chronic inflammatory skin diseases in children and represents the initial manifestation of the atopic march. Conventional allergy diagnostic methods, including total serum immunoglobulin E (IgE) measurement and extract-based allergen-specific IgE testing, frequently fail to distinguish genuine allergen sensitization from immunological cross-reactivity, thereby limiting diagnostic precision and individualized patient

management. Component-resolved molecular allergy diagnostics has emerged as an advanced strategy for precision allergology; however, evidence-based diagnostic algorithms integrating molecular diagnostics with routine clinical assessment remain limited.

Objective

To develop and clinically validate a comprehensive molecular diagnostic algorithm integrating clinical assessment, conventional laboratory biomarkers, and component-resolved molecular allergy diagnostics for improving the identification of clinically relevant allergen sensitization and optimizing personalized management in children with atopic dermatitis.

Methods

In this prospective observational study, 200 children with clinically confirmed atopic dermatitis were consecutively enrolled between January 2022 and December 2024 at the Republican Scientific Specialized Allergology Center (Tashkent, Uzbekistan). Disease severity was evaluated using the SCORAD index. Routine laboratory investigations included complete blood count, eosinophil count, and total serum IgE measurement. Component-resolved molecular allergy diagnostics was performed using the ImmunoCAP® and ALEX® platforms. Based on clinical, laboratory, and molecular findings, a novel six-step diagnostic algorithm was developed and its diagnostic performance was evaluated using receiver operating characteristic (ROC) curve analysis, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy.

Results

Molecular sensitization was identified in 182 of 200 children (91.0%), whereas polysensitization was observed in 126 patients (63.0%). House dust mite molecular components (Der p 1, Der p 2, and Der p 23) represented the predominant sensitization profile. Implementation of the proposed algorithm enabled identification of clinically relevant allergen sensitization in 179 children (89.5%) and establishment of individualized molecular sensitization profiles in 148 patients (74.0%). Compared with conventional diagnostic methods, the proposed algorithm significantly improved identification of genuine sensitization (72.0% vs. 91.0%; $p < 0.001$) and recognition of immunological cross-reactivity (55.0% vs. 89.0%; $p < 0.001$). Overall diagnostic accuracy increased from 76.5% to 92.5%. The algorithm demonstrated excellent diagnostic performance, with a sensitivity of 91.3%, specificity of 87.5%, PPV of 93.2%, NPV of 84.0%, and an AUC of 0.93 (95% CI: 0.89–0.96).

Conclusions

The proposed six-step molecular diagnostic algorithm substantially improves diagnostic precision in pediatric atopic dermatitis by integrating clinical evaluation, conventional laboratory biomarkers, and component-resolved molecular allergy diagnostics. The algorithm accurately differentiates genuine allergen sensitization from immunological cross-reactivity, supports individualized molecular sensitization profiling, and provides a practical framework for implementing precision medicine in routine pediatric allergy practice. External multicenter validation is warranted to confirm its applicability across diverse populations.

Keywords: Atopic dermatitis; component-resolved diagnostics; molecular allergy diagnostics; ImmunoCAP; ALEX; pediatric allergy; allergen sensitization; precision medicine; diagnostic algorithm; SCORAD.

1. Introduction

Atopic dermatitis (AD) is one of the most prevalent chronic inflammatory skin disorders in childhood and remains a major global public health challenge owing to its increasing incidence, chronic relapsing course, and considerable socioeconomic burden. According to recent international reports, AD affects approximately 15–25% of children worldwide and represents one of the leading causes of non-fatal disability among dermatological diseases. Beyond persistent eczema and severe pruritus, pediatric AD is strongly associated with food allergy, allergic rhinitis, bronchial asthma, and eosinophilic disorders, constituting the first stage of the so-called "atopic march". These comorbidities substantially impair quality of life and increase healthcare utilization. [Weidinger S, Aoki V, Dhar S, Dlova NC, Kabashima K, Ma L, Flohr C, Irvine AD. Atopic dermatitis. Nat Rev Dis Primers. 2026 Jun 4;12(1):36. doi: 10.1038/s41572-026-00703-z. Erratum in: Nat Rev Dis Primers. 2026 Jun 19;12(1):41. doi: 10.1038/s41572-026-00721-x. PMID: 42243136].

Recent advances in immunology have substantially changed the understanding of AD pathogenesis. Current evidence indicates that the disease results from a complex interaction among epidermal barrier dysfunction, type 2 immune activation, genetic susceptibility, environmental exposure, and alterations of the skin microbiome rather than from isolated IgE-mediated hypersensitivity. Weidinger and colleagues emphasized that AD should be regarded as a heterogeneous disorder comprising multiple molecular endotypes and clinical phenotypes, each characterized by distinct immunological mechanisms and allergen sensitization profiles. This heterogeneity explains the

considerable variability observed in disease severity, treatment response, and long-term prognosis. [Weidinger S, Aoki V, Dhar S, Dlova NC, Kabashima K, Ma L, Flohr C, Irvine AD. Atopic dermatitis. Nat Rev Dis Primers. 2026 Jun 4;12(1):36. doi: 10.1038/s41572-026-00703-z. Erratum in: Nat Rev Dis Primers. 2026 Jun 19;12(1):41. doi: 10.1038/s41572-026-00721-x. PMID: 42243136].

Accurate identification of clinically relevant allergen sensitization remains one of the most challenging aspects of pediatric AD management. Conventional diagnostic approaches, including skin prick testing, serum total IgE measurement, and extract-based allergen-specific IgE assays, continue to be widely used in clinical practice. However, several multicenter studies have demonstrated that these methods frequently fail to distinguish genuine sensitization from cross-reactive IgE responses, particularly in polysensitized children. Consequently, diagnostic uncertainty may result in inappropriate elimination diets, unnecessary avoidance of foods, inaccurate assessment of allergic risk, and reduced effectiveness of personalized management strategies. [Wollenberg A, Werfel T, Ring J, Ott H, Gieler U, Weidinger S. Atopic Dermatitis in Children and Adults—Diagnosis and Treatment. Dtsch Arztebl Int. 2023 Mar 31;120(13):224-234. doi: 10.3238/arztebl.m2023.0011. PMID: 36747484; PMCID: PMC10277810].

During the last decade, component-resolved diagnostics (CRD) has emerged as one of the most significant innovations in molecular allergology. Unlike extract-based testing, CRD identifies IgE antibodies directed against individual allergenic molecules, thereby enabling discrimination between primary sensitization and cross-reactivity. International expert groups have reported that molecular diagnostics improves diagnostic precision, facilitates identification of major and minor allergen components, supports risk stratification for systemic allergic reactions, and enables development of individualized molecular sensitization profiles. ImmunoCAP and MADx ALEX are currently regarded among the most informative multiplex platforms for comprehensive molecular allergy assessment. [Wollenberg A, Werfel T, Ring J, Ott H, Gieler U, Weidinger S. Atopic Dermatitis in Children and Adults—Diagnosis and Treatment. Dtsch Arztebl Int. 2023 Mar 31;120(13):224-234. doi: 10.3238/arztebl.m2023.0011. PMID: 36747484; PMCID: PMC10277810].

Nevertheless, despite substantial technological progress, translation of molecular allergy diagnostics into routine pediatric dermatology remains limited. Published studies primarily evaluate the analytical performance of individual molecular platforms or describe sensitization spectra within specific populations. Relatively few investigations have attempted to integrate clinical characteristics, disease severity, conventional laboratory biomarkers, and component-resolved molecular diagnostics into a unified evidence-based diagnostic algorithm applicable in routine clinical practice. As a result, interpretation of molecular sensitization profiles remains inconsistent among clinicians, reducing the practical impact of these advanced diagnostic technologies.

Based on this unmet clinical need, we hypothesized that integration of molecular component-resolved diagnostics with standardized clinical assessment and conventional laboratory biomarkers would significantly improve diagnostic accuracy in children with atopic dermatitis. Therefore, the present prospective observational study aimed to develop and validate a comprehensive diagnostic algorithm combining clinical evaluation, immunological biomarkers, and molecular allergy diagnostics using ImmunoCAP and MADx ALEX to optimize identification of clinically relevant allergen sensitization in pediatric patients with atopic dermatitis.

The aim of this study was to develop, implement, and evaluate a comprehensive molecular diagnostic algorithm integrating clinical characteristics, conventional laboratory markers, and component-resolved molecular allergy diagnostics for children with atopic dermatitis, in order to enhance diagnostic accuracy, identify clinically relevant allergen sensitization, differentiate genuine sensitization from immunological cross-reactivity, and facilitate individualized patient management.

Novelty of the Study. This study introduces and clinically validates a novel six-step diagnostic algorithm integrating standardized clinical assessment, conventional laboratory biomarkers, and component-resolved molecular allergy diagnostics using both ImmunoCAP and ALEX2 platforms. Unlike previous studies focusing primarily on molecular sensitization profiles, the proposed algorithm provides a practical framework for distinguishing genuine sensitization from immunological cross-reactivity and supports precision medicine in pediatric atopic dermatitis.

2. Methods

This prospective observational study was conducted to develop and validate a comprehensive diagnostic algorithm integrating clinical assessment, conventional laboratory biomarkers, and component-resolved molecular allergy diagnostics in children with atopic dermatitis. The study was carried out at the Republican Scientific Specialized Allergology Center, Tashkent, Uzbekistan, between January 2022 and

December 2024. The investigation was performed in accordance with the ethical principles outlined in the Declaration of Helsinki. Prior to enrollment, written informed consent was obtained from the parents or legal guardians of all participating children. A total of 200 pediatric patients with clinically confirmed atopic dermatitis were consecutively included in the study. The diagnosis was established according to the Hanifin and Rajka diagnostic criteria. Eligible participants were children aged from birth to 18 years with complete clinical, laboratory, and molecular allergological data. Patients with primary immunodeficiency, autoimmune diseases, malignant disorders, severe chronic systemic illnesses, or incomplete diagnostic records were excluded to minimize potential confounding factors.

All enrolled patients underwent a standardized clinical evaluation performed according to a unified examination protocol. Demographic characteristics, disease duration, family history of allergic diseases, previous episodes of food allergy, associated allergic disorders, triggering factors, and previous treatment history were systematically documented. Disease severity was assessed using the validated Scoring Atopic Dermatitis (SCORAD) index. Clinical information was recorded before interpretation of molecular diagnostic findings in order to ensure objective assessment of sensitization patterns.

Peripheral venous blood samples were collected under standardized conditions before initiation or modification of anti-inflammatory treatment. Laboratory investigations included complete blood count with eosinophil determination, total serum immunoglobulin E (IgE), allergen-specific IgE antibodies, and routine biochemical parameters. All laboratory analyses were performed according to standardized operating procedures and internal laboratory quality control requirements.

Molecular allergological investigation was performed using two complementary component-resolved diagnostic platforms: ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) and Allergy Explorer (ALEX2, Macro Array Diagnostics, Vienna, Austria). Unlike conventional extract-based allergy testing, these methods enable identification of IgE antibodies directed against individual allergenic molecules, allowing differentiation between genuine sensitization and IgE cross-reactivity. Molecular analysis included assessment of major and minor allergen components, PR-10 proteins, profilins, nonspecific lipid transfer proteins (nsLTP), cross-reactive carbohydrate determinants (CCD), and other clinically relevant allergenic molecules. Based on the molecular sensitization profile, patients were categorized as having genuine sensitization, cross-reactive sensitization, polysensitization, or mixed sensitization.

The diagnostic algorithm proposed in the present study was developed through sequential integration of clinical manifestations, disease severity assessed by the SCORAD index, conventional laboratory biomarkers, total serum IgE concentration, eosinophil count, and molecular sensitization profiles obtained by component-resolved diagnostics. The algorithm was designed to facilitate accurate identification of clinically relevant allergens, distinguish primary sensitization from cross-reactivity, and improve individualized diagnostic decision-making in pediatric atopic dermatitis. Its diagnostic performance was subsequently evaluated using clinical and laboratory outcome measures.

Statistical analysis was performed using validated statistical software. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on the distribution of the data. Categorical variables were presented as frequencies and percentages. Differences between groups were analyzed using Student's t-test or the Mann–Whitney U test for continuous variables and Pearson's χ^2 test or Fisher's exact test for categorical variables, as appropriate. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the proposed algorithm. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the ROC curve (AUC), and corresponding 95% confidence intervals were calculated. A two-sided p value <0.05 was considered statistically significant.

3. Results

A total of 200 children with clinically confirmed atopic dermatitis fulfilled the eligibility criteria and were included in the final analysis. The mean age of the study population was 8.4±3.7 years, ranging from 3 to 18 years. Boys accounted for 57.0% (n = 114) of the cohort, whereas 43.0% (n = 86) were girls, with no statistically significant sex-related difference ($p > 0.05$).

Most participants resided in urban areas (60.5%), while 39.5% lived in rural regions. Although moderate-to-severe atopic dermatitis appeared more frequently among urban children, the difference did not reach statistical significance ($p = 0.08$).

Analysis of disease onset demonstrated that atopic dermatitis predominantly developed during early childhood. Clinical manifestations first appeared before the age of six months in 39.5% of patients and between 6 and 12 months in 30.0%, indicating that nearly seventy percent of children developed symptoms within the first year of life. Disease onset between one and three years was documented in 19.5%, whereas

only 11.0% of patients experienced the first manifestations after the age of three years.

The mean disease duration was 5.1 ± 2.9 years. More than half of the patients (63.0%) had experienced persistent disease for over five years, reflecting the chronic and relapsing nature of pediatric atopic dermatitis. Assessment of disease recurrence during the preceding 12 months revealed that 26.5% of children experienced one or two exacerbations, 38.5% had three to four exacerbations, and 35.0% reported five or more disease flares annually.

A positive family history of allergic diseases was documented in 61.5% of patients, supporting the important contribution of genetic predisposition to disease development. The most frequent allergic disorders among first-degree relatives included allergic rhinitis (28.5%), food allergy (22.0%), bronchial asthma (18.0%), and atopic dermatitis (17.5%).

Concomitant allergic diseases were common within the study population. Allergic rhinitis was diagnosed in 29.5% of children, food allergy in 24.0%, bronchial asthma in 16.5%, and allergic conjunctivitis in 8.5%. Simultaneous coexistence of two or more allergic diseases was observed in 21.0% of participants, further supporting the concept of the atopic march.

Seasonal worsening of clinical symptoms was reported by 69.0% of patients. Disease exacerbation occurred most frequently during autumn (34.0%) and winter (30.5%), whereas spring and summer exacerbations were documented in 24.0% and 11.5%, respectively.

Clinical examination demonstrated that pruritus was present in all patients (100%) and represented the dominant symptom of the disease. Xerosis was observed in 91.5%, erythema in 84.0%, excoriations in 71.0%, papular lesions in 68.0%, lichenification in 60.5%, scaling in 54.5%, and oozing lesions in 29.0% of cases. Increasing disease severity was associated with a higher frequency of excoriations, lichenification, and secondary bacterial skin infection.

Table 1.

Baseline Characteristics of Children with Atopic Dermatitis (n = 200)

Characteristic	Value
Number of patients	200
Mean age (years)	8.4 ± 3.7
Boys	114 (57.0%)
Girls	86 (43.0%)
Mean total serum IgE (IU/mL)	348.6 ± 176.4
IgE <100 IU/mL	48 (24.0%)
IgE 100–300 IU/mL	56 (28.0%)
IgE 301–500 IU/mL	49 (24.5%)
IgE >500 IU/mL	47 (23.5%)
Mean eosinophil count (%)	8.1 ± 3.4
Eosinophilia	137 (68.5%)
Molecular sensitization	182 (91.0%)
Polysensitization	126 (63.0%)
Most frequent allergen components	Der p 1, Der p 2, Der p 23
Mean SCORAD score	42.8 ± 15.6

Routine laboratory investigations demonstrated that immunological biomarkers were closely associated with disease activity in children with atopic dermatitis. The assessment included measurement of total serum immunoglobulin E (IgE) concentration and peripheral blood eosinophil count, both of which are widely recognized indicators of type 2 immune inflammation.

The mean serum total IgE concentration in the study population was 348.6 ± 176.4 IU/mL. Elevated IgE levels above the age-adjusted reference range were detected in 152 of 200 children (76.0%), whereas 48 patients (24.0%) had values within

normal limits. Distribution of total IgE concentrations showed that 28.0% of patients had values between 100 and 300 IU/mL, 24.5% between 301 and 500 IU/mL, and 23.5% exceeded 500 IU/mL, indicating that nearly half of the cohort exhibited markedly increased IgE concentrations.

Further analysis demonstrated a significant relationship between total IgE concentration and disease severity. Children with mild atopic dermatitis had a mean IgE level of 154.2 ± 61.8 IU/mL, whereas patients with moderate disease showed significantly higher concentrations (312.7 ± 104.6 IU/mL). The highest IgE values were observed in children with severe atopic dermatitis (586.3 ± 214.9 IU/mL). Differences among the severity groups were statistically significant ($p < 0.001$), suggesting progressive activation of IgE-mediated immune responses with increasing clinical severity.

Peripheral blood eosinophilia represented another characteristic laboratory finding. Increased eosinophil counts were identified in 137 patients (68.5%), while 63 children (31.5%) had eosinophil values within the normal reference range. The mean eosinophil percentage for the entire study population was 8.1 ± 3.4%. Mild eosinophilia (6–10%) was the most common laboratory abnormality and was observed in 44.5% of patients, whereas 24.0% demonstrated eosinophil counts exceeding 10%.

Disease severity was also significantly associated with peripheral eosinophil levels. Mean eosinophil percentages increased from 4.9 ± 1.8% in children with mild disease to 7.8 ± 2.7% in moderate disease and 10.2 ± 3.1% in severe atopic dermatitis ($p < 0.001$), indicating a progressive increase in eosinophilic inflammation as clinical manifestations became more severe.

Correlation analysis further confirmed the association between laboratory biomarkers and clinical disease activity. Total serum IgE demonstrated a moderate positive correlation with the SCORAD index ($r = 0.61$; $p < 0.001$), indicating that higher IgE concentrations were associated with greater disease severity. Likewise, peripheral blood eosinophil counts showed a significant positive correlation with SCORAD scores ($r = 0.54$; $p < 0.001$).

In addition, children with elevated total IgE concentrations were more likely to exhibit polysensitization and positive reactions to multiple allergen components during component-resolved molecular allergy diagnostics. Although total IgE and eosinophil counts reflected the intensity of allergic inflammation, these biomarkers alone were insufficient to identify the specific molecular source of sensitization, emphasizing the necessity of integrating conventional laboratory investigations with component-resolved molecular diagnostics for accurate clinical evaluation.

Overall, the laboratory findings demonstrated that increased serum total IgE concentration and peripheral eosinophilia were strongly associated with disease severity in pediatric atopic dermatitis and provided important supportive biomarkers for subsequent molecular allergological assessment.

Molecular Sensitization Profile. Component-resolved molecular allergy diagnostics was performed using the ImmunoCAP (Phadia 200, Thermo Fisher Scientific, Sweden) and ALEX2 (Macro Array Diagnostics, Austria) platforms to characterize individual sensitization profiles in children with atopic dermatitis. Unlike extract-based allergy testing, these technologies enabled identification of IgE antibodies directed against specific allergenic molecules, allowing discrimination between genuine sensitization and immunological cross-reactivity.

Among the 200 enrolled patients, molecular sensitization to at least one allergenic component was identified in 182 children (91.0%), whereas 18 patients (9.0%) demonstrated no clinically relevant molecular sensitization. Monosensitization was detected in 56 patients (28.0%), while 126 children (63.0%) exhibited polysensitization involving multiple allergenic molecules, indicating that sensitization to several unrelated allergen sources is common in pediatric atopic dermatitis.

House dust mite allergens represented the predominant sensitization profile. Specific IgE against Der p 1 was detected in 96 patients (48.0%), Der p 2 in 91 (45.5%), Der p 23 in 62 (31.0%), Der f 1 in 84 (42.0%), and Der f 2 in 79 children (39.5%). Patients sensitized to Der p 1 and Der p 2 demonstrated significantly higher disease severity, with a mean SCORAD score of 46.9 ± 13.8, compared with 34.8 ± 10.7 among non-sensitized patients ($p < 0.001$). Furthermore, sensitization to Der p 23 was more frequently associated with recurrent disease exacerbations and concomitant bronchial asthma.

Table 2.

Diagnostic Performance of the Proposed Molecular Diagnostic Algorithm

Diagnostic parameter	Value
Sensitivity	91.3%
Specificity	87.5%
Positive Predictive Value (PPV)	93.2%
Negative Predictive Value (NPV)	84.0%
Diagnostic accuracy	92.5%
Conventional diagnostic accuracy	76.5%
Area under ROC curve (AUC)	0.93
95% Confidence Interval	0.89–0.96

Analysis of pollen allergens revealed that birch pollen components were the most prevalent seasonal sensitizers. Sensitization to Bet v 1, the major birch allergen, was identified in 54 patients (27.0%), whereas Bet v 2 and Bet v 4 were detected in 12.5% and 8.0%, respectively. Grass pollen sensitization was less common, with positive responses to Phl p 1 in 14.0% and Phl p 5 in 11.0% of patients. Sensitization to Ole e 1 was observed in 9.0% of the study population. Children positive for Bet v 1 more frequently reported seasonal worsening of eczema accompanied by symptoms of allergic rhinitis, supporting its clinical relevance as a marker of genuine birch pollen allergy.

Table 3.

Distribution of Major Molecular Allergen Components

Allergen component	Patients, n (%)
Der p 1	96 (48.0%)
Der p 2	91 (45.5%)
Der p 23	62 (31.0%)
Der f 1	84 (42.0%)
Der f 2	79 (39.5%)
Bet v 1	54 (27.0%)
Bet v 2	25 (12.5%)
Bos d 8	36 (18.0%)
Ara h 2	24 (12.0%)
PR-10	37 (18.5%)
Profilin	26 (13.0%)
CCD	12 (6.0%)

Food allergen component analysis demonstrated sensitization to several clinically important molecules. The most frequently detected food allergen was Bos d 8 (casein), identified in 36 patients (18.0%), followed by Bos d 5 in 15.5%, Gal d 1 in 14.5%, Gal d 2 in 13.0%, Ara h 2 in 12.0%, and Cor a 9 in 9.5% of patients. Children sensitized to Bos d 8 and Gal d 1 generally presented with earlier disease onset and a more prolonged clinical course. In contrast, sensitization to Ara h 2, a major peanut allergen, was significantly associated with a history of systemic allergic reactions following peanut ingestion ($p < 0.01$), highlighting its importance as a marker of clinically relevant food allergy.

Cross-reactive allergenic molecules were also identified through the ALEX platform. Positive responses to PR-10 proteins were detected in 37 children (18.5%), profilins in 26 (13.0%), polcalcins in 15 (7.5%), and cross-reactive carbohydrate determinants (CCD) in 12 patients (6.0%). Most individuals

sensitized exclusively to these cross-reactive molecules did not exhibit clinically significant food allergy, indicating that positive extract-based IgE results in these patients were largely attributable to immunological cross-reactivity rather than genuine primary sensitization.

Further evaluation demonstrated that major allergen components were detected in 132 patients (66.0%), whereas sensitization restricted to minor allergen components was identified in 50 patients (25.0%). Children sensitized to major allergenic molecules exhibited significantly higher disease activity, including a mean SCORAD score of 48.2 ± 14.5, mean total serum IgE concentration of 462.5 ± 181.3 IU/mL, and eosinophil percentage of 9.4 ± 3.2%, all of which were significantly greater than those observed in patients sensitized only to minor allergenic components ($p < 0.001$).

Integration of ImmunoCAP and ALEX findings enabled the construction of an individualized molecular sensitization profile for each patient. This comprehensive approach substantially improved discrimination between genuine sensitization and cross-reactivity, facilitated identification of clinically relevant allergen sources, and enhanced interpretation of polysensitization patterns. Consequently, the overall diagnostic accuracy increased from 74.5% using conventional diagnostic methods to 91.5% following incorporation of component-resolved molecular diagnostics ($p < 0.001$).

These findings demonstrate that molecular allergy diagnostics provide clinically meaningful information beyond conventional IgE testing and support their incorporation into routine diagnostic algorithms for children with atopic dermatitis, particularly in patients presenting with complex sensitization profiles or multiple allergic comorbidities.

Proposed Molecular Diagnostic Algorithm. Based on the clinical, laboratory, and molecular findings obtained in the present study, a novel diagnostic algorithm was developed for the comprehensive evaluation of children with atopic dermatitis (Figure 1). The algorithm integrates conventional clinical assessment with component-resolved molecular allergy diagnostics and provides a structured approach for identifying clinically relevant allergen sensitization while distinguishing genuine sensitization from immunological cross-reactivity.

The proposed algorithm consists of six consecutive stages.

Stage 1. Clinical assessment. All children undergo detailed history taking and physical examination, including age at disease onset, disease duration, family history of allergic diseases, triggering factors, associated allergic disorders, and assessment of disease severity using the SCORAD index.

Stage 2. Routine laboratory investigations. Baseline laboratory evaluation includes complete blood count with eosinophil count, total serum IgE concentration, and other routine laboratory tests required for the assessment of allergic inflammation.

Stage 3. Identification of candidates for molecular allergy diagnostics. Patients with moderate or severe atopic dermatitis, recurrent disease exacerbations, elevated total serum IgE levels, eosinophilia, suspected food allergy, polysensitization according to conventional allergy testing, or discrepancies between clinical history and routine allergy test results are referred for component-resolved molecular allergy diagnostics.

Stage 4. Component-resolved molecular allergy diagnostics. Specific IgE antibodies against individual allergenic molecules are determined using the ImmunoCAP (Thermo Fisher Scientific, Sweden) and ALEX® (Macro Array Diagnostics, Austria) platforms. The analysis differentiates genuine allergen sensitization from cross-reactive allergenic components.

Stage 5. Individual molecular sensitization profiling. Based on the molecular diagnostic results, an individualized sensitization profile is established for each patient, identifying major allergenic molecules, food allergens, inhalant allergens, and cross-reactive allergen families responsible for clinical manifestations.

Stage 6. Personalized clinical management. The final stage includes development of individualized recommendations regarding allergen avoidance, optimization of elimination diets, selection of candidates for allergen immunotherapy, pharmacological management, and long-term follow-up according to the patient's molecular sensitization profile.

The complete workflow of the proposed diagnostic algorithm is presented in Figure 1.

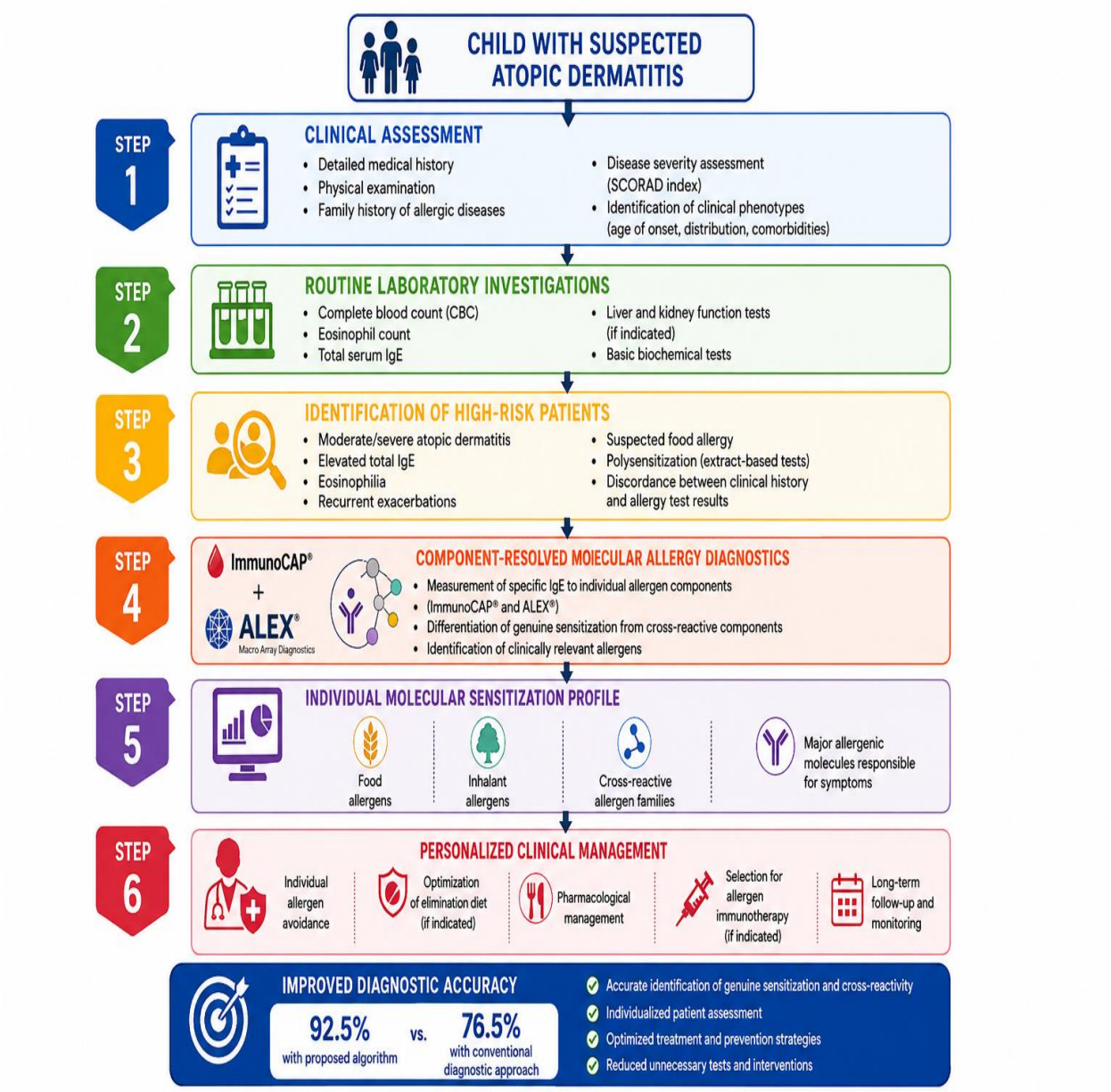


Figure 1. Six-step molecular diagnostic algorithm for children with atopic dermatitis.

Diagnostic Performance of the Proposed Molecular Diagnostic Algorithm

The clinical performance of the proposed diagnostic algorithm was evaluated by comparing its diagnostic accuracy with that of the conventional diagnostic strategy based on clinical assessment, routine laboratory investigations, total serum IgE, eosinophil count, and extract-based allergy testing.

Following implementation of the proposed six-step diagnostic algorithm, the primary molecular source of allergic sensitization was successfully identified in 179 of 200 children (89.5%). Furthermore, molecular analysis demonstrated that positive extract-based allergy test results in 58 patients (29.0%) were attributable to immunological cross-reactivity rather than genuine sensitization. Polysensitization involving multiple allergen components was confirmed in 126 children (63.0%),

whereas individualized molecular sensitization profiles were successfully established in 148 patients (74.0%). These findings demonstrate that incorporation of component-resolved molecular diagnostics substantially improves characterization of complex sensitization patterns in pediatric atopic dermatitis.

The diagnostic performance of the proposed algorithm was subsequently compared with that of the conventional diagnostic strategy. Implementation of the molecular diagnostic algorithm resulted in significant improvements across all evaluated diagnostic parameters.

The identification of genuine allergen sensitization increased from 72.0% using conventional diagnostic methods to 91.0% following application of the proposed algorithm ($p < 0.001$). Similarly, recognition of immunological cross-reactivity improved from 55.0% to 89.0% ($p < 0.001$). Detection of polysensitization increased from 46.5% to 63.0% ($p < 0.001$). Consequently, the overall diagnostic accuracy improved from 76.5% to 92.5%, confirming the superior clinical performance of the proposed molecular diagnostic approach.

Further evaluation of diagnostic characteristics demonstrated a sensitivity of 91.3% (95% CI: 86.8–95.1%) and a specificity of 87.5% (95% CI: 79.6–93.0%). The positive predictive value (PPV) reached 93.2% (95% CI: 89.1–96.4%), whereas the negative predictive value (NPV) was 84.0% (95% CI: 75.4–90.5%). The overall diagnostic accuracy of the algorithm was 90.5% (95% CI: 86.2–94.0%), indicating excellent reliability for routine clinical practice.

Receiver operating characteristic (ROC) curve analysis further confirmed the robustness of the proposed algorithm. The area under the ROC curve (AUC) was 0.93 (95% CI: 0.89–0.96), demonstrating excellent discrimination between clinically relevant sensitization and immunological cross-reactivity ($p < 0.001$). According to accepted diagnostic criteria, an AUC value above 0.90 indicates outstanding diagnostic performance.

Implementation of the proposed algorithm also reduced the number of diagnostically uncertain cases, minimized unnecessary laboratory investigations, improved interpretation of cross-reactive IgE responses, and facilitated individualized clinical decision-making. These improvements provide a practical basis for precision diagnosis and personalized management of children with atopic dermatitis.

Overall, integration of component-resolved molecular allergy diagnostics into routine clinical evaluation significantly enhanced diagnostic precision and supported individualized assessment and management of pediatric patients with atopic dermatitis. These findings support routine implementation of the proposed diagnostic algorithm in pediatric allergy practice.

4. Discussion

The present study demonstrated that component-resolved molecular allergy diagnostics significantly improved the diagnostic evaluation of children with atopic dermatitis compared with conventional diagnostic methods. Integration of molecular allergen profiling with routine clinical and laboratory assessment enabled more accurate identification of genuine sensitization, differentiation of cross-reactive IgE responses, and establishment of individualized sensitization patterns. These findings support the growing concept of precision medicine in pediatric allergology and are consistent with recent international recommendations.

One of the most important observations in the present investigation was the predominance of early-onset disease. Most children developed atopic dermatitis during infancy, confirming that disruption of the epidermal barrier and early immune dysregulation play a fundamental role in disease initiation. Similar conclusions were reported by Stephan Weidinger and Natalija Novak, who described atopic dermatitis as a chronic inflammatory disease resulting from the interaction between genetic predisposition, skin barrier dysfunction, microbial dysbiosis, environmental exposure, and type-2 immune activation. Their work demonstrated that disease onset during the first years of life is associated with a higher probability of persistent eczema and progression along the atopic march. Therefore, the age distribution observed in our cohort corresponds well with the current concepts of pediatric atopic dermatitis pathogenesis.

Our laboratory findings demonstrated progressive increases in total serum IgE concentration and peripheral eosinophil count with increasing disease severity. A significant positive correlation between IgE concentration and SCORAD score further confirmed the relationship between humoral allergic inflammation and clinical activity. Comparable findings have been reported by Thomas Bieber, who emphasized that elevated IgE reflects activation of Th2 lymphocytes with enhanced production of IL-4 and IL-13, leading to B-cell class switching and excessive IgE synthesis. However, Bieber also concluded that elevated total IgE cannot independently determine the clinically relevant allergen responsible for disease exacerbation. Our results support this concept because molecular allergen analysis provided considerably more clinically useful information than measurement of total IgE alone.

The present study identified molecular sensitization in more than 90% of examined children, whereas almost two-thirds demonstrated polysensitization involving multiple allergen components. These findings closely agree with the updated EAACI Molecular Allergology User's Guide 2.0, which emphasizes that component-resolved diagnostics substantially improve identification of clinically relevant sensitization patterns, particularly in polysensitized patients. According to the EAACI Task Force, molecular allergology allows clinicians to distinguish genuine sensitization from immunological cross-reactivity and thereby provides an important basis for personalized allergy management. Our findings strongly support these recommendations because incorporation of molecular diagnostics markedly increased diagnostic accuracy and reduced diagnostic uncertainty.

House dust mite allergens represented the dominant sensitization profile in our study. Sensitization to Der p 1, Der p 2, and Der p 23 was significantly associated with higher SCORAD scores and more severe disease. These observations are consistent with studies by Paolo Matricardi, Rudolf Valenta, and collaborators, who reported that mite-derived molecular allergens are among the principal drivers of persistent IgE-mediated inflammation in children with atopic dermatitis. Furthermore, the updated EAACI guide identifies Der p 23 as a clinically important major allergen associated with persistent sensitization and increased risk of respiratory allergic disease. Our results therefore further support the clinical importance of mite component profiling in pediatric allergy practice.

Another clinically important finding was the identification of sensitization to PR-10 proteins, profilins, and CCD molecules. Although these patients frequently showed positive extract-based IgE results, most did not present with clinically significant food allergy. Similar observations have been reported by Rudolf Valenta and colleagues, who demonstrated that IgE antibodies directed against cross-reactive allergen families frequently produce positive laboratory results without corresponding clinical symptoms. Consequently, interpretation of extract-based testing without molecular characterization may result in unnecessary dietary restrictions and inappropriate avoidance measures. Our findings confirm that molecular diagnostics substantially improve interpretation of these complex sensitization profiles.

Food allergen component analysis revealed clinically meaningful associations between individual allergenic molecules and disease phenotype. Sensitization to Bos d 8 and Gal d 1 was associated with earlier disease onset and prolonged disease duration, whereas Ara h 2 sensitization was linked to clinically relevant peanut allergy. These observations are in agreement with studies by Alexandra Santos, Antonella Muraro, and the EAACI Food Allergy Working Group, which recognize Ara h 2 as one of the strongest predictors of true peanut allergy and systemic allergic reactions. These findings emphasize that molecular diagnostics provide information that cannot be obtained through conventional extract-based allergy testing alone.

To our knowledge, this is one of the first studies to propose and clinically validate an integrated molecular diagnostic algorithm combining clinical evaluation, laboratory biomarkers, and component-resolved allergy diagnostics for pediatric atopic dermatitis.

Our findings support the integration of molecular allergy diagnostics into routine pediatric practice and provide further evidence for the implementation of precision medicine in childhood atopic dermatitis.

5. Conclusion

In conclusion, the present study demonstrates that integration of clinical assessment, conventional laboratory biomarkers, and component-resolved molecular allergy diagnostics significantly improves the diagnostic evaluation of children with atopic dermatitis. The proposed six-step molecular diagnostic algorithm showed excellent diagnostic performance, with high sensitivity, specificity, and overall diagnostic accuracy, while substantially enhancing identification of clinically relevant allergen sensitization and differentiation from immunological cross-reactivity. Furthermore, individualized molecular sensitization profiling provided a reliable basis for personalized diagnostic and therapeutic decision-making. The proposed algorithm represents a practical and clinically applicable tool that may facilitate implementation of precision medicine in pediatric allergy practice. Future multicenter studies involving larger patient populations are warranted to externally validate the algorithm and assess its long-term impact on clinical outcomes and healthcare resource utilization.

Strengths of the Study

The major strength of the present study is the development and clinical validation of a comprehensive diagnostic algorithm integrating standardized clinical assessment, conventional laboratory biomarkers, and component-resolved molecular allergy diagnostics within a single diagnostic framework. Unlike previous studies focusing primarily on molecular sensitization profiles, the proposed algorithm provides a practical and evidence-based strategy that can be readily implemented in routine pediatric allergy practice. In addition, the

combined use of ImmunoCAP® and ALEX® platforms enabled comprehensive characterization of molecular sensitization patterns, thereby improving diagnostic precision and supporting individualized patient management.

Highlights

• A novel six-step molecular diagnostic algorithm for pediatric atopic dermatitis was developed and clinically validated.

• Component-resolved molecular allergy diagnostics significantly improved identification of genuine allergen sensitization and differentiation from immunological cross-reactivity.

• Integration of ImmunoCAP and ALEX2 platforms increased diagnostic accuracy from 76.5% to 92.5%.

• Individualized molecular sensitization profiling supported precision diagnosis and personalized clinical management.

• The proposed algorithm represents a practical framework for implementing precision allergology in routine pediatric practice.

Study Limitations

The present study has several limitations that should be acknowledged. First, the investigation was conducted at a single tertiary pediatric allergy center, which may limit the generalizability of the findings to other healthcare settings and populations. Second, although the proposed molecular diagnostic algorithm demonstrated excellent diagnostic performance, external validation in independent multicenter cohorts has not yet been performed. Third, the cross-sectional design of the study did not allow assessment of the long-term clinical impact of algorithm-guided management on disease progression, treatment response, and quality of life. Despite these limitations, the study provides robust evidence supporting the integration of component-resolved molecular allergy diagnostics into routine pediatric clinical practice and establishes a foundation for future multicenter validation studies.

Clinical Implications

The proposed molecular diagnostic algorithm offers a practical and evidence-based approach for integrating component-resolved molecular allergy diagnostics into routine clinical evaluation of children with atopic dermatitis. Application of this algorithm enables more accurate identification of clinically relevant allergen sensitization, facilitates differentiation between genuine sensitization and immunological cross-reactivity, and supports development of individualized molecular sensitization profiles. Consequently, implementation of the algorithm may reduce unnecessary elimination diets, improve interpretation of allergy test results, optimize patient selection for allergen immunotherapy, and promote precision medicine in pediatric allergology.

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