# Diagnostic approaches for differentiating between true fruit allergy and oral allergy syndrome

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## ABSTRACT

*The purpose of the present study was to identify diagnostic markers that would allow distinguishing true food allergy to fruits from oral allergy syndrome, as well as to evaluate the efficacy of various diagnostic approaches. The study included 182 adult patients from Poland with immediate allergic reactions after consuming fresh fruits. Diagnosis was conducted in stages and included the collection of clinical data, allergy testing with fruit and pollen allergens, laboratory determination of total and specific immunoglobulin E (IgE), molecular methods of sensitisation identification, and assessment of cell activation. A comparative analysis of skin tests and specific immunoglobulin E determination revealed a 69.2% overall concordance, but diagnostic discrepancies were noted in more than 20% of cases, highlighting the need for a comprehensive approach. The use of component-resolved diagnostics helped to identify sensitisation to individual stable protein structures that are not accessible with standard testing and to clarify the form of allergy – true or cross-reactive. The data obtained demonstrated the need to integrate molecular diagnostics and functional methods into clinical practice to improve the accuracy of the differential diagnostics of fruit allergy.*

***Keywords:*** Cellular reactivity, cross-sensitisation, molecular diagnostics, thermolabile proteins.

## INTRODUCTION

Food allergy to fruits is a pressing issue in clinical allergology, considering its widespread prevalence, diverse clinical manifestations, and the complexity of differential diagnostics. It is particularly challenging to distinguish between true allergy and oral allergy syndrome (OAS), which is a form of allergic reaction primarily triggered by cross-reactions between pollen allergens (such as birch or grass pollen) and proteins found in certain fresh fruits. While OAS typically presents as localized symptoms, such as itching, swelling, and burning in the mouth, true fruit allergies can cause more severe, systemic reactions, including skin rashes, gastrointestinal distress, and respiratory symptoms. This distinction is crucial for effective diagnosis and treatment, as OAS is often triggered by heat-labile proteins, with patients tolerating cooked fruits, while true food allergies are not dependent on the fruit's preparation. The problem of cross-reactivity between pollen and food allergens is particularly pronounced in regions with a high prevalence of pollen sensitisation (Bekov *et al.*, 2023; Pashova *et al.*, 2019). Research found that the structural similarity between the main birch allergen Bet v 1 and its homologues in apples, cherries, peaches, and other fruits is the cause of symptoms that occur in patients after consuming these products. Knyziak-Mędrzycka *et al.* (2024) investigated the molecular structure of homologous proteins and concluded that it is thermolability and sensitivity to gastric enzymes that determine the limited clinical manifestations of cross-reactive oral allergy.

The clinical variability of fruit allergy manifestations requires the development of reliable diagnostic algorithms that include both primary medical history and laboratory and challenge tests. Kuźniar *et al.* (2024) analysed the effectiveness of skin tests in patients with suspected fruit allergy and concluded that low standardisation of extracts and the influence of

concomitant pollen sensitisation reduce the reliability of the method. However, the researchers did not consider the possibility of component diagnosis, which limits the practical applicability of their conclusions.

Modern methods of component-resolved diagnostics enable the identification of specific protein components associated with severe reactions (Alimardanova *et al*., 2015; Uikassova *et al*., 2022). De Rose *et al.* (2021) applied a molecular approach to the differentiation of fruit allergies and demonstrated high sensitivity in the detection of stable components such as lipid transfer proteins and profilins. Functional tests, such as the basophil activation test (BAT), allow assessing the reactivity of blood cells to allergen contact in vitro. This allows confirming the clinical significance of the detected Immunoglobulin E (IgE) antibodies. Yoshida *et al.* (2022) conducted a BAT study in patients with borderline serology results and found that the method can serve as a reliable tool in doubtful cases. Double-blind placebo-controlled food challenge (DBPCFC) tests continue to be the gold standard for confirming food allergies, despite their complexity and risks. Haidar *et al.* (2024) conducted a large-scale study of the effectiveness of DBPCFC in patients with suspected fruit allergy and found that reproducible symptoms were observed in only some patients, with a considerable proportion of reactions being mild.

Thus, despite the availability of various diagnostic methods, the problem of differentiating between OAS and true allergy is still unresolved. The development of a diagnostic approach based on a stepwise evaluation of clinical symptoms, immunological markers, and functional test results appears necessary. The purpose of the study was to identify diagnostic criteria for differentiating between true food allergy to fruit and OAS and to evaluate the sensitivity and specificity of the methods used.

## MATERIALS AND METHODS

The study was conducted in Poland between January and December 2023 at the allergology department of the University Medical Centre in Warsaw, with the participation of the private diagnostic centre MedGen, which specialises in molecular allergology. The study included 182 patients aged 18 to 65 years (74 men and 108 women), with an average age of 34.6±12.1 years. All participants complained of itching, burning, and swelling of the mucous membranes of the mouth and lips, as well as skin and gastrointestinal symptoms after consuming fresh fruit. Notably, all subjects had a history of pollinosis symptoms, primarily during the spring and summer. The cohort demonstrated a predominance of women (59.3%) compared to men (40.7%)

The initial clinical stage included a standardised questionnaire based on the European Academy of Allergy and Clinical Immunology (EAACI) questionnaire (Gromek *et al.*, 2024), a physical examination, and skin prick tests with allergens from birch, hazel, artemisia, and the 12 most commonly consumed fruits using diagnostic extracts (Stallergenes Greer, France). To assess sensitisation, total and specific IgE levels were determined using the Immuno CAP method (Thermo Fisher Scientific, Sweden). Particular attention was paid to the components Bet v 1, Mal d 1, Cor a 1, Pru p 1, Act d 1, and Ara h 8. In cases where conventional tests were insufficiently informative, component-resolved analysis was performed using Allergens Evaluation by Multiplex Diagnostics (ALEX2) (MacroArray Diagnostics, Austria), which enables quantitative and qualitative assessment of sensitisation to more than 280 allergens and their protein components. In 17 patients, a BAT was additionally performed by flow cytometry using Flow Cytometry Equipment (BD Biosciences, USA), which supported the clinical significance of sensitisation at the cellular level.

In 38 patients with conflicting data, a series of oral provocation tests (DBPCFC) was performed following the EAACI protocol (Santos *et al.*, 2025) using lyophilised fruits (in doses of 5-100 g) and placebo, administered at 20-minute intervals. Patients were kept under constant monitoring in a day hospital equipped with anti-shock measures. The Sampson severity grading scale was used to assess the severity of the reaction.

Statistical data processing was performed using Statistica 13.3 (TIBCO Software Inc.,

USA). For quantitative variables, the Student's t-test or Mann-Whitney U-test was employed, and for categorical variables, the chi-square test or Fisher's exact test was used. Multivariate logistic regression analysis was used to identify predictors of true allergy. Differences were considered significant at $p<0.05$. The study followed the Declaration of Helsinki (1964) and the principles of good clinical practice; all participants signed informed consent forms.

## RESULTS AND DISCUSSION

### Clinical manifestations and symptom profiles

Most often, patients reported symptoms occurring within the first 15 minutes after eating fresh fruit. The most typical manifestations were itching and swelling of the lips and palate (89.6%), a burning sensation in the mouth (76.4%), and itching in the throat (71.4%). Skin reactions in the form of urticaria or localised itching were observed in 41.2% of patients, and dyspeptic disorders in 24.2%. All patients had a history of seasonal allergic rhinitis, mainly in the spring and summer, with 68.7% associating the worsening of symptoms with the birch flowering season. It was noted that in more than half (52.7%) of patients, reactions occurred exclusively to raw fruits, while heat-treated fruits did not cause symptoms, which already at this stage suggested a possible association with thermolabile allergens (Table 1).

At the same time, the presence of skin manifestations in the form of urticaria and itching outside the orofacial area in 41.2% of patients, as well as gastrointestinal symptoms in 24.2% (Table 1), indicates a broader spectrum of clinical reactions that do not always fit within the framework of a localised form of OAS. These manifestations can be considered as potential markers of systemic hypersensitivity and require further evaluation with specific tests. Notably, all patients had a history of seasonal allergic symptoms, confirming the pollen-based sensitisation. It is particularly significant that 68.7% of patients associated the exacerbation of symptoms with the spring period, which indicates a possible role of cross-reactivity, although at this stage this remained a hypothesis that was yet to be corroborated by laboratory tests.

Interestingly, 52.7% of the subjects reported the development of symptoms exclusively after eating raw fruits (Table 1), while heat-treated products were tolerated without discomfort. This observation is an essential clinical indicator of differences in the thermal stability of allergens, but without laboratory verification, it does not allow a definitive conclusion to be drawn about the nature of the sensitisation.

### Diagnostic approaches and results

A comparative evaluation of the results of skin testing and the determination of specific IgE levels in the blood serum of the patients examined revealed a general trend of high frequency of sensitisation to fruits using both methods. The most frequent sensitisation was to apples, kiwis, and peaches. Despite the significant level of agreement between the two methods, discrepancies were noted in a series of cases, requiring further interpretation. The summary data is presented in Table 2.

### Frequency of positive skin tests and specific IgE to fruits

Analysis of the diagnostic data presented in Table 2 demonstrated prominent features of sensitivity and diagnostic concordance of the two basic approaches – skin prick testing and determination of specific IgE levels in blood serum – in the examination of patients with suspected allergy to fresh fruits. Skin tests performed using standard extracts revealed sensitisation in a significant proportion of patients. The greatest number of positive reactions was recorded with the introduction of apple extract (63.7%), followed by kiwi (44.5%) and peach (41.2%). This supported that these fruits were the most medically significant allergens in the study cohort. For cherries and plums, the rates were significantly lower – 27.5% and 21.4%, respectively, which could suggest both a lower prevalence of sensitisation and potentially weaker allergenic activity of the extracts used in the tests.

In parallel with the skin tests, specific IgE levels were determined, which showed slightly lower overall sensitivity. Thus, IgE to apple was detected in 58.2% of patients, to kiwi – in 39.0%, to peach – in 36.3%, to cherry – in 22.5%, and to plum – in 19.2% (Table 2).

Although the quantitative differences between the methods were not radical, they were systematic and suggested that skin testing more often revealed reactivity, possibly due to a local effect and greater skin sensitivity to allergenic extracts.

The concordance between skin test results and serological data varied depending on the particular allergen. The greatest level of concordance was observed for apple (72.5%), which was probably explained by the good representation of apple allergenic components in both skin test extracts and the laboratory analysis panel. For peaches and kiwis, the concordance rates were 66.2% and 61.5%, respectively, while for cherries and plums – 58.9% and 60.1%, respectively (Table 2). This data suggested that even when using standard diagnostic panels, discrepancies between in vivo and in vitro methods are possible, especially with less typical fruits.

Cases of discrepancies between the results of the two methods deserved special attention. In 11.5% of patients, skin tests were positive with negative specific IgE values (Table 2). This situation could be conditioned by skin hypersensitivity, the inclusion of non-specific irritants in the extract, individual characteristics of the immune response, or low antibody titres that did not reach the diagnostic threshold in laboratory analysis. In contrast, 9.3% of the subjects showed the opposite situation: detection of IgE antibodies with negative skin tests (Table 2). In these cases, the possible influence of antihistamine use, reduced skin reactivity, or characteristics of the allergen poorly represented in the prick test used were significant. The overall concordance between the two methods was 69.2% (Table 2), which, on the one hand, suggested an acceptable level of diagnostic convergence, but on the other hand, highlighted the existence of diagnostic "blind spots" covering over 20% of cases.

**Results of component diagnostics (ALEX2) and basophil activation test**

Refined diagnostics using component-resolved methods and cell tests were performed in patients in whom basic methods (skin tests and specific IgE determination) did not allow for the unambiguous establishment of clinically relevant sensitisation. The ALEX2 test was used in 56 patients with inconclusive results, while the BAT was used in 17 patients, mainly with severe symptoms but no laboratory confirmation. These methods enabled a more accurate determination of allergen profiles and establishment of the clinical significance of previously unidentified sensitisations. The summarised results are presented in Table 3.

The use of component-resolved diagnostics involving the ALEX2 multiplex platform in 56 patients with inconclusive or conflicting results from previous diagnostic stages substantially improved the understanding of their sensitisation profile. In 85.7% of cases (48 patients), positive results were obtained, i.e., sensitisation to concrete protein components of allergens that could not be detected by conventional tests was identified. This associated with the high sensitivity of the method and its ability to detect "hidden" sensitisation that is not accessible using extract-based tests. In 14.3% of cases (8 patients), the component test was negative, which corresponded to clinically mild or questionable symptoms in these individuals (Table 3).

The performance of BAT in 17 patients, predominantly with borderline IgE levels and no clinically verified skin reactions, helped to further assess the functional activity of sensitising allergens. A positive BAT result was obtained in 82.4% of cases (14 patients), and all those patients had previously experienced symptoms caused by the consumption of particular fruits in their daily lives. This confirmed that the detected IgE was clinically significant and caused the activation of effector cells. In the remaining 3 cases (17.6%), BAT was negative in the presence of specific IgE, which allowed classifying the sensitisation as subclinical or cross-reactive, with no prognostic value (Table 3). This refers to the role of IgE in triggering a functional immune response, as evidenced by positive BAT results, which indicate that the immune system actively responded to allergens. In cases where BAT was negative, sensitization was considered functionally irrelevant, indicating that it did not contribute to clinical symptoms and therefore had no prognostic significance.

**Advanced diagnostic techniques and clinical implications**

DBPCFC tests were performed in 38 patients with ambiguous results of skin testing, specific IgE, and/or component diagnosis. All tests were performed in a day hospital setting, with sequential administration of lyophilised fruits and placebo at 20-minute intervals. Reactions were recorded using the Sampson severity grading scale. A positive response, defined as the reproduction of typical symptoms after administration of the active substance, was recorded in 21 patients (55.3%). In 17 cases (44.7%), the provocation tests did not cause any symptoms, which allowed ruling out the clinically relevant allergy. Most of the positive reactions occurred within the first 15-30 minutes and were limited to mild orofacial manifestations. Systemic reactions developed in 6 cases (15.8%), including skin, respiratory, and gastrointestinal symptoms. No patient experienced anaphylaxis or life-threatening conditions. Placebo did not cause reactions in any participant, confirming the specificity of the responses. Positive reactions were most frequently observed with the administration of freeze-dried apples (42.8%), peaches (28.5%), and kiwis (23.8%). The summary data are presented in Table 4.

DBPCFC tests in 38 patients with conflicting results from previous diagnostic stages helped to reliably determine the clinical significance of sensitisation and finally classify the type of allergic reaction. A positive response was recorded in 21 patients (55.3%), suggesting the presence of a reproducible reaction to fruit components under controlled exposure conditions (Table 4). Orofacial manifestations dominated – itching, swelling of the lips and mucous membranes, and a burning sensation, which corresponded to the typical clinical picture of OAS. However, 6 patients (15.8%) experienced systemic reactions, including skin rashes, dyspeptic symptoms, and respiratory symptoms (Table 4). These cases were considered clinically significant true food allergies requiring exclusion of the corresponding product from the diet and consideration of allergen-specific immunotherapy. Thus, DBPCFC played a key role in verifying the diagnosis, especially in cases where laboratory and skin tests gave ambiguous or inconclusive results.

Analysis of the distribution of positive reactions by fruit type showed the highest frequency of medically significant sensitivity to apples (42.8% of all positive responses), followed by peaches (28.5%) and kiwis (23.8%) (Table 4). These data correlated with the results of skin and serological tests, where these fruits also showed the greatest frequency of sensitisation. Thus, apples supported their significance as the leading allergen in the study population. Notably, no cases of anaphylaxis or severe life-threatening conditions were reported. Negative tests recorded in 44.7% of the subjects allowed ruling out the clinically relevant allergy and suggested that the previously identified sensitisation had no pronounced clinical significance.

The presented study identified key clinical features inherent in patients with suspected oral allergy syndrome or true food allergy to fresh fruits. The established predominance of localised symptoms – itching and swelling of the mucous membranes of the mouth and lips (89.6%), burning sensation in the mouth (76.4%), and itching in the throat (71.4%) – was consistent with the typical manifestation of OAS. This is confirmed by data from Gromek *et al.* (2024) and Guvenir *et al.* (2024), who described an analogous symptom profile in patients sensitised to pollen allergens. Combined with the patients' reports of worsening symptoms in the spring and summer, these factors associated with a high probability of cross-reactivity between pollen and food proteins. However, the detection of skin reactions (41.2%) and dyspeptic symptoms (24.2%) broadens the clinical picture beyond classic OAS. Analogous findings were also reported by Şirin *et al.* (2024), who emphasised the possibility of systemic manifestations in patients with latent true food allergy. Thus, the present study demonstrated the existence of a wide range of symptoms requiring a stratified diagnostic approach. The fact that 52.7% of the subjects examined had reactions exclusively to raw fruits suggests the involvement of heat-labile allergens, which is consistent with the findings of Schmitt (2024).

Diagnostic evaluation based on a comparison of skin prick tests and specific IgE levels demonstrated a satisfactory level of agreement (69.2%), especially for apples (72.5%). This confirms the high clinical significance of this fruit, which was also reflected in studies by Torres-Arroyo *et al.* (2024). However, the discrepancies between the methods (in 20.8% of cases) highlighted the diagnostic limitations of each, especially towards less typical allergens. Cases of positive skin tests with negative IgE results and vice versa require separate analysis. Barni *et al.* (2022) also described analogous diagnostic discrepancies, attributing them to individual characteristics of the immune response and variability in the allergenic composition of extracts.

The use of ALEX2 in patients with inconclusive results of standard tests helped to identify the specific sensitisation in 85.7% of cases, demonstrating the high effectiveness of this method in clarifying allergic status. Apart from the ability of ALEX2 to detect previously undiagnosed sensitisation, especially to individual protein structures, a valuable advantage is the ability to distinguish between primary sensitisation and cross-reactions. Terlouw *et al.* (2024) obtained analogous findings, highlighting the high sensitivity of the method and its ability to differentiate between primary and cross-sensitisation. Furthermore, the multiplex test format enables the simultaneous detection of a wide range of allergens, which expedites diagnostics and reduces the need for multiple tests.

Of particular significance is the detection of sensitisation to thermolabile proteins – specifically, recombinant homologues of Bet v 1 – in patients who react only to raw fruits. Such proteins are homologues of the major birch pollen allergen and rapidly denature under the influence of temperature, which explains the lack of reaction to heat-treated products (Petrenko *et al*., 2022). This allows Bet v 1-like structures to be considered key markers of cross-allergy between pollen and fruits (Pashova and Radev, 2021; Shahini *et al*., 2023). These data are consistent with the findings of Wang *et al.* (2024), who described analogous molecular mechanisms in pollen-food syndrome, pointing to a high prevalence of sensitisation to these proteins in populations with pollen rhinoconjunctivitis.

The BAT performed in 17 patients showed positive results in 82.4% of cases and associated with the clinical significance of previously detected IgE. Corresponding data were presented by Shah *et al.* (2023) emphasising the high specificity of BAT in assessing the functional activity of allergens. In the present study, BAT supported its diagnostic value in situations where serological and skin tests gave conflicting results. However, Skypala *et al.* (2022) questioned the advisability of routine use of BAT in allergological practice, pointing to its excessive cost and limited reproducibility.

DBPCFC tests allowed confirming the diagnosis definitively in complex clinical cases. The data obtained (55.3% positive responses) correlated with the findings of Costa and Mafra (2022), who emphasised the need to use DBPCFC as the “gold standard” for diagnosing food allergies. The predominance of mild reactions in most of the subjects corroborated the dominance of OAS. However, the presence of systemic manifestations in 15.8% of patients requires special attention, as the spread of symptoms beyond the orofacial area may suggest a more pronounced degree of sensitisation and a potential risk of developing severe allergic reactions.

The results of the study have significant practical implications for clinical allergology. Component-specific diagnosis, in particular the ALEX2 method, is useful in cases where standard methods, such as skin tests or specific IgE determination, give ambiguous or contradictory results. It allows for the accurate determination of sensitization to specific protein components of allergens, which is important for differentiating between cross-reactivity and true allergy. The use of the BAT is particularly indicated for patients with borderline IgE levels and no clinically confirmed skin reactions, as it helps to assess the functional activity of sensitizing allergens. At the same time, traditional methods such as skin tests and IgE determination remain important tools in diagnosis, especially for the general assessment

of sensitization to the most common allergens, such as fruits.

## CONCLUSIONS

The presented study of the clinical and diagnostic characteristics of 182 patients with suspected allergic reactions to fresh fruit found that the most widespread symptoms were itching and swelling of the mucous membranes of the mouth and lips (89.6%), burning in the mouth (76.4%), and itching in the throat (71.4%), which corresponded to the typical clinical picture of oral allergy syndrome. The average age of the patients was 34.6 years, with a predominance of females (59.3%). All patients had a history of seasonal allergic rhinitis, and 68.7% associated the exacerbation of symptoms with birch flowering, indicating a high probability of pollen-food cross-sensitisation. At the same time, the presence of systemic symptoms (in 15.8% with Double-blind placebo-controlled food challenge), skin manifestations (41.2%), and dyspepsia (24.2%) suggested a more heterogeneous nature of reactions that extended beyond the scope of classic oral allergy syndrome.

The combined use of skin tests and serological analyses (specific IgE) helped to identify the allergen-specific sensitisation in most patients, with apples, kiwis, and peaches being the most significant allergens. The concordance between the two methods reached 69.2%, but discrepancies (over 20%) revealed the limitations of each approach. Component-resolved diagnostics refined the sensitisation profile in 85.7% of patients with ambiguous results, identifying clinically significant IgE to both heat-labile and heat-stable proteins. Functional assessment using the basophil activation test showed positive results in 82.4% of subjects, confirming the biological activity of the detected antibodies. Double-blind placebo-controlled food challenge tests finally supported the diagnosis in patients with borderline results from previous diagnostics. The study found that more than half of the reactions were limited to mild symptoms characteristic of oral allergy syndrome, while some patients had systemic manifestations indicating true food allergy.

## CONFLICT OF INTEREST STATEMENT

The author declare that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Table 1: Clinical profile of study participants**

| Indicator | Value |
|---|---|
| Total number of patients | 182 |
| Male/Female | 74/108 (40.7%/59.3%) |
| Average age (years±SD) | 34.6±12.1 |
| Itching and swelling of the mucous membrane of the mouth/lips | 89.6% |
| Burning sensation in the mouth | 76.4% |
| Itching in the throat | 71.4% |
| Skin reactions (hives, itching) | 41.2% |
| Gastrointestinal symptoms | 24.2% |
| Seasonal allergic rhinitis in medical history | 100% |
| Worsening of symptoms during birch blossoming | 68.7% |
| Reaction only to raw fruit | 52.7% |

**Table 2: Frequency of positive skin tests and specific IgE to fruits**

| Allergen (fruit) | Positive skin tests (%) | Positive IgE (%) | Coincidence of results (%) |
|---|---|---|---|
| Apples | 63.7 | 58.2 | 72.5 |
| Kiwis | 44.5 | 39.0 | 61.5 |
| Peaches | 41.2 | 36.3 | 66.2 |
| Cherries | 27.5 | 22.5 | 58.9 |
| Plums | 21.4 | 19.2 | 60.1 |
| Overall consistency | - | - | 69.2 |
| Divergence (skin+/IgE–) | - | - | 11.5 |
| Divergence (IgE+/skin–) | - | - | 9.3 |

**Table 3: Results of component diagnostics (ALEX2) and basophil activation test**

| Diagnostic procedure | Number of patients | Positive results | Negative results | Clinical significance corroborated (%) |
|---|---|---|---|---|
| ALEX2 | 56 | 48 (85.7%) | 8 (14.3%) | 85.7% |
| BAT | 17 | 14 (82.4%) | 3 (17.6%) | 82.4% |

**Table 4: Results of double-blind food challenge tests with fruits**

| Indicator | Value |
|---|---|
| Number of patients | 38 |
| Positive response | 21 (55.3%) |
| Negative response | 17 (44.7%) |
| Systemic responses | 6 (15.8%) |
| Anaphylaxis | 0 (0%) |
| Placebo responses | 0 (0%) |
| Most reactive fruits | Apples (42.8%), peaches (28.5%), kiwis (23.8%) |