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E-Issn 2845-7285

Volume 58 N. 2/2026 – March 2026

European Annals of Allergy and Clinical Immunology

THE OFFICIAL JOURNAL OF AAIITO | ASSOCIAZIONE ALLERGOLOGI IMMUNOLOGI ITALIANI TERRITORIALI E OSPEDALIERI

THE OFFICIAL JOURNAL OF SPAIC | SOCIEDADE PORTUGUESA DE ALERGOLOGIA E IMUNOLOGIA CLINICA



2/2026

2023 Journal Impact Factor: 2.6



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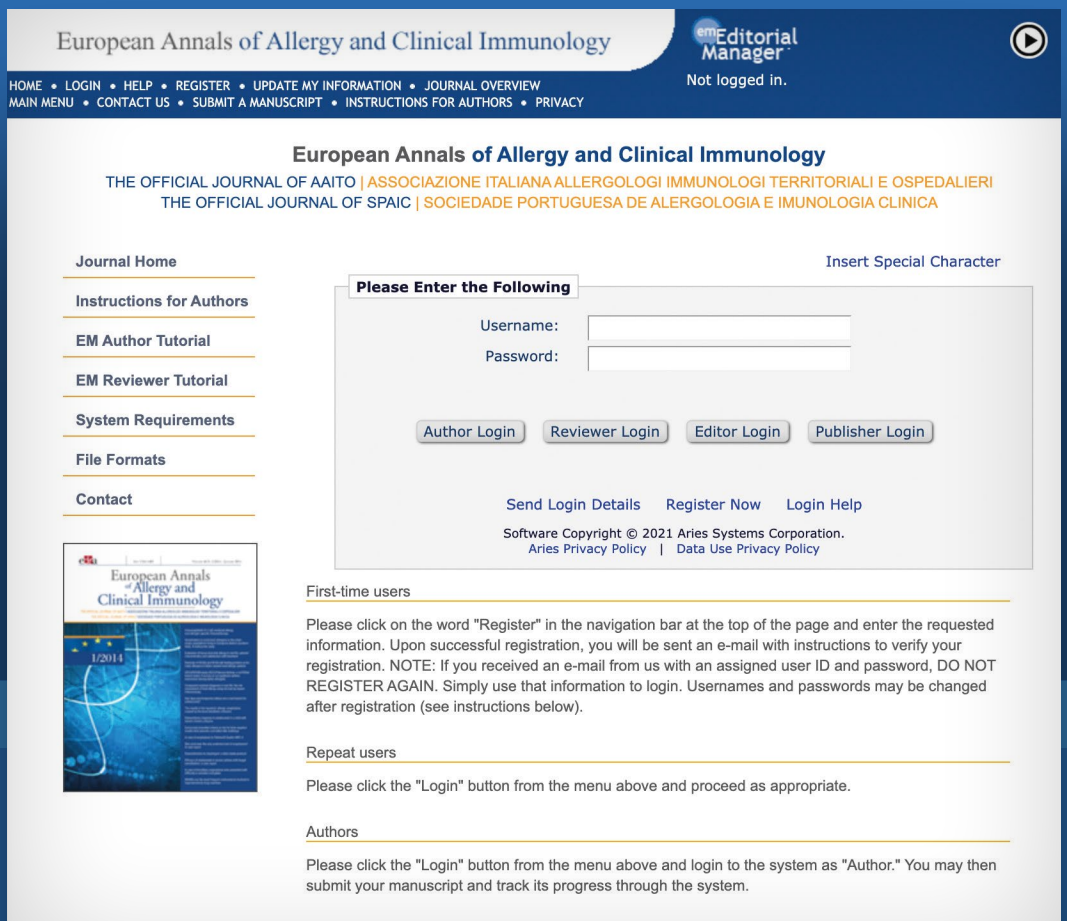
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Viale Forlanini, 21
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www.edizioniedra.it

"European Annals of Allergy and Clinical Immunology" registered at Tribunale di Milano - n. 336 on 22.10.2014

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edra MEDIA REVIEW | Eur Ann Allergy Clin Immunol | Vol 58, N.2, 51-60, 2026

Maria Zofia Lisiecka

Allergic reactions to spices: a review of sensitivities to pepper, cumin, oregano, anise, mustard and other spices

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Key words

Cross-reactivity; pollen-food syndrome; anaphylaxis; PR-10; Bet v 1.

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Doi
10.23822/EurAnnACI.1764-1489.400

Impact statement

Spice allergies pose serious health risks, causing symptoms from skin rashes to anaphylaxis. Improved diagnostics and treatments are essential to manage this often-overlooked allergy effectively.

Summary

Spice allergies are often under-recognized and under-researched, leading to delays in diagnosis and treatment. Cross-reactivity with other plant allergens further complicates accurate diagnosis. This literature review seeks to systematize and analyze current data on hypersensitivity to spices, including pepper, cumin, oregano, anise, mustard, and other seasonings. The review covers research published from 2010 to 2023 in peer-reviewed journals, books, and conference proceedings, sourced from databases such as PubMed, Scopus, Web of Science, and Google Scholar. The main pepper allergens are PR-10 proteins, profilins and defensins. They can cause cross-allergic reactions with pollen and other plant allergens. Cumin allergens belong to the PR-10 family and can provoke allergic reactions. Cross-reactivity between cumin and other umbelliferae is common. Oregano contains Bet v 1 allergens and profilins, often leading to cross-allergies with other spices and plants. Allergies to anise are less common but can be a serious problem due to cross-reactivity with birch pollen. Mustard is one of the most allergenic spices. These proteins can cause severe reactions even in small amounts. Diagnosis is carried out using skin prick tests and blood tests for specific IgE antibodies. Cross-reactivity among spices and other allergens complicates the diagnosis and management of spice allergies. This review will be useful for the development of personalized dietary recommendations for patients, accounting for cross-reactivity and individual sensitization profiles.

Introduction

Allergic reactions to spices are an urgent problem in modern allergology. Spices such as pepper, cumin, oregano, anise, mustard and many others are widely used in cooking around the world. Their applications range from home cooking to mass food production. Despite their popularity and usefulness, spices can cause allergic reactions in sensitive people, which makes studying this problem extremely important.

Following Kanikowska *et al.* (1) from 2022, the prevalence of food allergies varies depending on the age group: in children, it is estimated at 6-10%, while in adults this figure is 2-5%. In addition, according to a study by Mazur *et al.* (2), about 20% of people suffering from food allergies, in addition to gastrointestinal symptoms, also have atopic dermatitis. This emphasizes the relationship between food allergies and skin diseases such as atopic dermatitis. Krzych-Falta *et al.* (3) demonstrated significant variability in the prevalence of food allergy and the importance of individual allergens in different parts of the world. In Europe, the percentage of people reporting food allergies ranges from 1.7% to 37.3%, depending on the country. In North America, this figure ranges from 3.1% to 11%. The problem with the study is that allergic reactions to spices often remain underestimated and insufficiently studied. This can lead to delays in diagnosis and treatment, as well as underestimation of their prevalence and severity. Allergic reactions to spices can manifest themselves in the form of skin rashes, angioedema, gastrointestinal symptoms and even anaphylaxis (4). Such symptoms can significantly impair the quality of life of patients and require serious medical intervention.

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Previously, both domestic and foreign scientists were engaged in research in this area. For instance, Sikorska-Szaflik and Sozańska (5) studied the risk factors for food allergy, among which he noted genetic predisposition, epigenetic changes and the impact of environmental factors that can play an important role in the onset and development of this disease. Food additives were studied and, thus, the incidence of allergic reactions to food additives was determined to be less than 1% of all food hypersensitivities in adults and to be about 2% in children (6). However, despite significant progress in this area, many unresolved issues remain. For instance, it is still unclear how spices cause allergic reactions at the molecular level and which spice components are the main allergens. In addition, more detailed data is needed on the prevalence of spice allergy in different age and ethnic groups (7). Methods of diagnosing and preventing spice allergy are also insufficiently studied, especially in the context of mass production and consumption of food.

The study systematizes and analyses existing data on sensitivity to various spices, including pepper, cumin, oregano, anise, mustard and others. Author plans to review the mechanisms of allergic reactions, clinical manifestations, diagnostic methods and treatment options for spice allergy. Special attention will be paid to the prevalence of this problem and possible methods of prevention.

Materials and methods

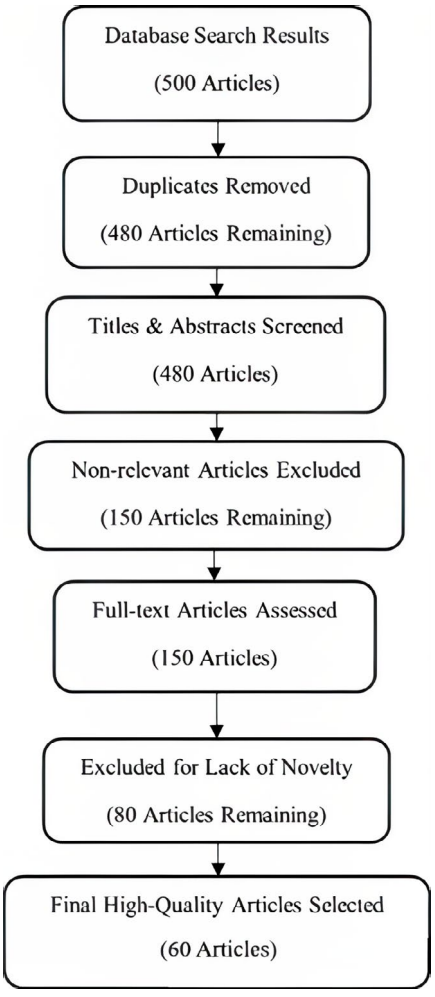
To perform this literature review, a comprehensive assessment of existing studies on allergic reactions to spices such as pepper, cumin, oregano, anise and mustard was conducted. The review covers the period from 2010 to 2023 and includes publications from peer-reviewed scientific journals, books and conference proceedings. The main sources of data were PubMed, Scopus, Web of Science and Google Scholar databases.

The inclusion criteria were as follows: publications devoted to allergic reactions to the specified spices; studies describing clinical manifestations of allergy to spices; works on the mechanisms of allergic reactions to spices; articles containing methods of diagnosing allergy to spices; publications describing approaches to the treatment and prevention of allergy to spices; works in Russian and English; peer-reviewed studies published in scientific journals. The exclusion criteria were as follows: studies not related to the topic of spice allergy; papers that have not been peer-reviewed; publications that do not contain new data or have no scientific novelty; duplicate publications already included in the analysis; articles written in languages other than Russian and English.

The literature search was carried out using keywords and their combinations, such as "spice allergy", "pepper allergy", "cumin allergy", "oregano allergy", "anise allergy", "mustard allergy", "food allergy", "diagnosis of spice allergy", "treatment of spice allergy". Both general and specialized terms, as well as synonyms and medical terms, were used to ensure the completeness of the search. Additionally, author used advanced combinations, including different forms and declensions of keywords to cover the maximum number of relevant publications. For instance, variations of "pepper allergy", "allergic reactions to cumin", "oregano sensitivity", "anise allergy", "immunoreaction to mustard", and others were used. Medical terms and synonyms were also considered, such as "*Capsicum* allergy" for pepper allergy, "*Cuminum cyminum* sensitivity" for cumin allergy, "*Origanum vulgare* allergy" for oregano allergy, and "*Pimpinella anisum* allergy" for anise allergy. The use of scientific Latin names of plants was used to find additional research that could have been missed if author had used only commonly used terms.

The stages of the literature review included several steps. In the first stage, an initial search was conducted using keywords in databases, which resulted in more than 500 articles and publications. Then, based on the abstracts and keywords, the publications that

Figure 1 *- Flowchart of article selection process for spice allergy review.*



met the inclusion criteria were selected. At this stage, about 150 articles were selected. The full texts of the selected articles were carefully studied to determine their relevance to the topic of the review. After this stage, 80 articles remained for detailed analysis. Further, the data was systematized into the following categories: clinical manifestations of allergy, mechanisms of allergic reactions, diagnostic methods, approaches to treatment and prevention. Each article was analyzed to identify key data and then integrated into the review. To assess the quality and reliability of the data, author used the criteria for evaluating peer-reviewed publications, including methodological aspects, sample size, control groups, statistical significance of the results and sample representativeness. At the last stage, 60 of the most relevant and high-quality publications were finally selected from the remaining 80 articles to be included in the review. All relevant data was synthesized into a single analytical overview presented in a structured manner. Key topics and areas for future research were identified. All ethical norms and rules were upheld. All works were properly cited with authors and sources of information. **Figure 1** provides a visual summary of the stages involved in this process.

Results and discussion

Pepper allergy and the role of capsaicin

Pepper allergy is a common phenomenon that can cause a wide range of symptoms in sensitive people. The main allergenic components of pepper are proteins such as PR-10, profilins and defensins. In a study from 2023, Bochorishvili *et al.* (8) showed that out of 435 allergic patients examined, 38% (164 patients) demonstrated reactivity to pan allergens of the PR-10 family, profilins and lipid transport proteins. Among them, PR-10 was the most common allergen with a sensitization rate of 56%, followed by profilins with 43% and lipid transport proteins with 32%. Sensitization to PR-10 allergens has been associated with allergic rhinitis and asthma. Continuing with the topic of allergenic proteins, a 2023 review by Barre *et al.* (9) characterized PR-10, profilins and lipid transport proteins as the main allergenic proteins in fruit. The review determined that most fruit allergens belong to these families, confirming their importance in cross-allergic reactions. These proteins can cause cross-allergies with pollen and other plant allergens, such as spices, plant pollen and others. Black pepper, red pepper, and cayenne pepper can cause allergic reactions in sensitive individuals (9, 10). These proteins can trigger a strong immune response in people with a predisposition to allergies. In a 2021 study by Kattupalli *et al.* (11), a genomic analysis of PR-1 proteins from black pepper was conducted. They identified 11 PR-1 genes that play a critical role in protecting plants from biotic and abiotic stresses. PR-1 genes are involved in the plant's defense mechanisms against *Phytophthora capsici*, a pathogen that causes root rot. This study showed a significant level of PR-1 gene expression. Perić *et al.* (12) determined that an allergic reaction to black pepper usually occurs after prolonged exposure to this allergen, which was demonstrated by the example of allergic rhinitis in an employee of a black pepper production plant. In addition, a study from 2022 by Takei *et al.* (13) identified *Capsicum annuum* allergens, including Cap a 7, which shows cross-reactivity with Japanese cedar and peach pollen allergens. In a patient sensitive to these allergens, IgE reactivity to Cap a 7 and other proteins was found, which emphasizes the importance of these allergens in allergic reactions to red pepper.

Furthermore, in a study from 2021, Ivens *et al.* (14) investigated the cross-reactivity of chili peppers with Brazil nuts and hazelnuts using the xMAP food allergen test. They determined a high degree of cross-reactivity between chili and nut allergens, which emphasizes the complexity of allergen analysis in spices and the need to consider cross-reactivity in the diagnosis of allergies. Lastly, in a study conducted by Wróblewska *et al.* (15), the immunoreactivity of proteins in Capsicum (pepper) spices was investigated. Using mass spectrometry and in silico analysis, the researchers identified several highly immunoreactive allergenic proteins, including Cap a 1, Cap a 2 and Cap a 7, which can cause severe allergic reactions. The study found hidden allergens and contaminants that could pose a health risk to sensitive people.

Symptoms of allergic reactions to pepper can range from mild skin rashes and itching to serious respiratory problems and ana-

Table I *- Brief description of allergens.*

Spice	Prevalence of allergies	Clinical manifestations
Pepper	38%	Hives, swelling of the lips and throat, respiratory problems, anaphylaxis
Cumin	20%	Skin rash, itching, nasal congestion, anaphylaxis
Oregano	Unknown	Cross-allergy, skin rashes, gastrointestinal symptoms
Anise	Rarely	Cross-allergy with birch pollen, skin rashes
Mustard	High	Severe allergic reactions, urticaria, anaphylaxis

phylactic shock. The most common manifestations are hives, swelling of the lips and throat, difficulty breathing, nausea and vomiting (**table I**).
In some cases, gastrointestinal disorders, such as abdominal pain and diarrhea, are observed. These symptoms can occur either immediately after consuming pepper or after a period, making it difficult to diagnose an allergy.

Caraway allergy and the role of Bet v1
Cumin is a popular spice widely used in various cuisines around the world. However, it can cause allergic reactions in some people. Cumin allergens belong to the PR-10 family and can provoke allergic reactions (16). These proteins can cause allergic reactions in sensitive people, leading to various allergic symptoms. In a 2021 review, Singh *et al.* (17) studied the phytochemistry and pharmacology of cumin. They noted that the main bioactive component of cumin is cuminaldehyde, which can cause allergic reactions, and its interaction with other spices requires further study. Clinical manifestations of a caraway seed allergy can include skin symptoms such as hives, itching and swelling, as well as respiratory problems such as nasal congestion, sneezing and difficulty breathing (18, 19). Some people also experience gastrointestinal disorders such as nausea, vomiting and abdominal pain. These symptoms can occur both immediately after consuming cumin and after a period of time, which makes it difficult to diagnose an allergy. The severity of the symptoms can range from mild manifestations to severe systemic reactions, including anaphylactic shock (20).
Cross-reactivity of cumin with other spices and allergens is common. This is due to the presence of similar protein structures in different plants. People with cumin allergy can also react to dill, parsley, carrots and other members of the Umbelliferae family. Studies have shown that the similarity in protein structures between cumin and other plants can lead to cross-allergy, which is confirmed by the work of El-Rady *et al.* (21). It is worth noting that cross-reactivity can make it difficult to diagnose and manage allergies, as patients may experience symptoms when eating different foods. In the course of Słowianek *et al.* (22) research, new allergens were found in caraway seeds, including Bet v 1 analogue, profilin and elongation factor α. Furthermore, Asero *et al.* (23) observed that systemic reactions and gastrointestinal symptoms were the most frequent clinical manifestations of allergy to spices from the Piperaceae family. Importantly, systemic reactions to PR-10 proteins, such as Bet v 1, are generally associated with the use of proton pump inhibitors, which may influence allergenic responses by altering gastric digestion and immune sensitization. Treatment of cumin allergy is designed around avoidance of triggers and symptomatic treatment. Patients are advised to exclude cumin and potentially cross-reactive foods from their diet. In the event of severe allergic reactions such as anaphylactic shock, patients should have epinephrine auto-injectors available for immediate use. In addition, Li *et al.* (24) determined the reason for the cross-reaction of cumin allergy with other allergens, the main allergen of birch pollen is Bet v 1, one of the seven recognized allergens. Component-specific diagnostics help to distinguish true birch allergy from false positives caused by pan-allergens. Patients with symptoms of birch pollen allergy have elevated levels of IgE to Bet v 1, which also indicates the risk of developing oral allergy syndrome when eating fruits of the Rosaceae family, nuts and vegetables of the Apiaceae family. Allergies to birch (*Betula*) and cereal (*Poaceae*) pollen can contribute to the development of an allergic reaction to oregano due to the similarity of allergenic proteins. From 2010 to 2015 in Davos and Munich, Maya-Manzano *et al.* (25) measured airborne pollen and its allergens Bet v 1 and Phl p 5. Most of the allergens were found in the PM>10 fraction. The Pollen Allergen Potency (PAP) for cereals was significantly higher in Munich than in Davos, but there was no difference for birch. PAP varied by day, year and location, increasing with the season for Poaceae, but remaining constant for *Betula*. Bet v 1 is also present in cumin, which causes a cross-reaction. Yasudo *et al.* (26) also notes that sensitization to Bet v 1 in pollen allergy is a risk factor for spice allergy.
In conclusion, cumin allergy is a significant problem that requires careful diagnosis and an individual approach to treatment. Research in the field of molecular allergology and cross-reactivity helps to better understand the mechanisms of allergic reactions and develop effective methods of diagnosis and treatment. Raising awareness among healthcare professionals and patients about the potential for allergic reactions to spices, including cumin, and developing new therapeutic approaches can significantly improve allergy management and quality of life.

Oregano allergy and the role of profilins in its development
Oregano, a popular spice in Mediterranean and Mexican cuisine, can also cause allergic reactions in some people. The main allergenic proteins of oregano are Bet v 1 and profilin, which belong to the PR-10 and profilin families. These proteins are the main triggers of the immune response in sensitive individuals, leading to a variety of allergic symptoms. Clinical manifestations of an oregano allergy can include skin symptoms such as hives, itching and swelling, as well as respiratory problems including nasal congestion, sneezing and difficulty breathing. Some people may also experience gastrointestinal symptoms such as nausea, vomiting and abdominal pain (27). In rare cases, anaphylactic shock may occur, requiring immediate medical attention. In a study conducted to comprehensively assess the sensitization profile to food allergens in Polish children, the lowest sIgE sensitization rates to food allergen extracts were found for oregano (0.3%). This emphasizes the rarity of allergic reactions to oregano among the children in the study (28).
The link between oregano allergy and other food allergies is well understood. People with oregano allergy often also react to

Table II *- Cross-activity of spices.*

Spice	Diagnostic methods	Approaches to treatment	Cross-reactivity
Pepper	Skin prick tests, blood tests for IgE	Exclusion from the diet, adrenaline auto-injectors for severe cases	With pollen and other plant allergens
Cumin	Skin prick tests, blood tests for specific IgE antibodies	Exclusion from the diet, symptomatic treatment	With dill, parsley, carrots
Oregano	Blood tests for specific IgE antibodies	Dietary elimination, antihistamines	With other spices and herbs
Anise	Blood tests for specific IgE antibodies	Dietary elimination, antihistamines	With birch pollen

other spices such as basil, rosemary and sage, as well as to certain fruits and vegetables such as banana, kiwi, tomato and pepper. This cross-reactivity is due to the similarity of protein structures between the different plants (**table II**).

Studies show that people with oregano allergies may experience symptoms when eating other foods containing similar allergens. A study by Wagner *et al.* (29) in 2022 found that 29% of patients with allergies to birch, wormwood or grass pollen, had positive skin tests for herbs, including oregano. This emphasizes the importance of considering sensitization to herbs in the diagnosis of food allergy. In a 2023 review, Fernandes *et al.* (30) investigated the antimicrobial properties of plants in the Lamiaceae family. They found that plants such as rosemary have strong antimicrobial properties but can also cause allergic reactions in sensitive people. A study by Högerle *et al.* (31) showed that patients with birch allergy, who also have specific IgE to the components of the allergen Bet v 1 and profilin, are more likely to suffer from oral allergic syndrome and intestinal reactions. They also have more frequent and severe symptoms of rhinitis and asthma. In conclusion, Poncet *et al.* (32) report that 30 to 60% of patients with food allergies also suffer from cross-reactions with plant pollen, which is explained by the fact that food allergens in spices and pollen have a similar structure and belong to the same family of proteins PR10, as well as profilins.

Diagnosis of oregano allergy is usually carried out using skin prick tests or blood tests for specific IgE antibodies. Skin prick tests can detect immediate allergic reactions to oregano and other potentially cross-reactive foods. Blood tests for specific IgE antibodies help confirm the diagnosis and determine the level of sensitization to oregano. In some cases, additional tests, such as provocation tests, may be necessary to clarify the diagnosis. Treatment of oregano allergy consists of eliminating oregano and cross-reactive foods from the diet. Patients are advised to read food labels carefully and avoid eating foods containing oregano and other potentially hazardous ingredients. In case of allergic reactions, antihistamines are used to relieve symptoms such as itching, hives and nasal congestion. In more severe cases, corticosteroids or epinephrine may be required to control anaphylactic reactions. In conclusion, oregano allergy is a significant problem for some people, especially those living in regions where this spice is widely used. Careful diagnosis and effective management of oregano allergy can significantly improve the quality of life of patients, allowing them to avoid adverse reactions and minimize the risk of serious complications.

Hypersensitivity to anise and its related species

Allergic reactions to anise are less common than to other spices, but can still be a serious problem for some people. Bet v 1 proteins and profilins belong to the PR-10 and profilin families, respectively. These proteins trigger an immune response in sensitive individuals, leading to various allergic symptoms. In most cases, no allergic reaction is observed directly to anise, but it is most often reacted to by people with birch pollen allergy as part of a cross-reaction (33). Polak *et al.* (34) reported that in areas where birch predominates, allergy to pollen of Fagales trees is often initiated by the allergen Bet v 1, which is the cause of cross-allergy with anise and other spices.

The prevalence of anise allergy varies by region and population. In the Mediterranean countries, where anise is widely used in cooking and medicine, allergic reactions are more common than in other regions. A study by Wang *et al.* (35) in Northern China showed that the main allergen of birch pollen, Bet v 1, is the leading component causing sensitization, with a level of 82.8%. Pollen food allergy syndrome (PFAS) was identified in 75.9% of participants, of whom 72.7% were allergic to apples. Significantly higher levels of sIgE to birch pollen and Bet v 1 were observed in patients with PFAS and apple allergy. Bet v 1 proved to be a valuable biomarker for the diagnosis of PFAS and apple allergy, while Bet v 2 should also be considered in immunotherapy.

Clinical manifestations of anise allergy can include skin symptoms such as hives, itching and swelling, as well as respiratory problems including nasal congestion, sneezing and difficulty breathing (36). Some people may also experience gastrointestinal symptoms such as nausea, vomiting and abdominal pain. In rare cases, anaphylactic shock may occur, requiring immediate medical attention (37). In addition to the main reactions, Younis *et al.* (38) provides evi-

dence that plasma cell gingivitis (PCG) can occur as one of the possible reactions to anise. PCG is an inflammatory condition affecting the mucous membrane of the oral gums, characterized by dense polyclonal infiltration of plasma cells in the connective tissue (39, 40). The etiology of PCG is related to hypersensitivity to certain antigens, such as toothpaste, mouthwashes, chewing gums and spices, including anise. Complications and risks of anise allergy include the development of anaphylactic shock, which can be life-threatening. Therefore, it is important to recognize allergy symptoms promptly and take appropriate precautions. Early diagnosis and proper management of aniseed allergy can significantly reduce the risk of severe reactions and improve the quality of life of patients.

According to the findings of the study by Beutner *et al.* (41), skin prick tests or blood tests for specific IgE antibodies are commonly used to diagnose anise allergy. Skin prick tests can detect immediate allergic reactions to anise and other potentially cross-reactive products. Blood tests for specific IgE antibodies help to confirm the diagnosis and determine the level of sensitization to anise. In some cases, additional tests, such as provocation tests, may be necessary to clarify the diagnosis. As an example, Cacheiro-Llaguno *et al.* (42) used prick tests, which revealed that the most common molecular allergen was Bet v 1 (84%).

In conclusion, anise allergy is a significant problem for some people, especially those living in regions where this spice is widely used. Careful diagnosis and effective management of anise allergy can significantly improve the quality of life of patients, avoiding adverse reactions and minimizing the risk of serious complications.

Allergic reactions to mustard

Mustard is one of the most allergenic spices due to the presence of strong allergens such as Sin a 1, Sin a 2, Sin a 3 and Sin a 4. These proteins belong to the 2S albumin family and can cause serious allergic reactions even in small amounts (43). Assou *et al.* (44) described the allergen Bra j, the removal of which with the help of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPS) allowed a significant reduction of the allergenicity of mustard. Mustard allergy can manifest itself in various symptoms, including skin, respiratory and food reactions.

Sin a 2, Sin a 3, and Sin a 4 are potent allergens derived from mustard, exhibiting distinctive characteristics that augment their allergenic potential. Sin a 2, which belongs to the 11S globulin family, is highly stable and resistant to digestion. This allows it to trigger robust immune responses and even to survive food processing. This protein frequently exhibits cross-reactivity with other 11S globulins present in nuts and legumes, thereby increasing the likelihood of allergic reactions in individuals with prior exposure to mustard allergens. Sin a 3, a non-specific lipid transfer protein (nsLTP), is also highly allergenic and has the potential to elicit severe reactions, including anaphylaxis, even at trace amounts. Furthermore, it exhibits notable cross-reactivity with nsLTPs in fruits such as peaches, apples, and grapes, frequently resulting in oral allergy syndrome (OAS). Although less extensively researched, Sin a 4 belongs to the profilin family and exhibits comparable cross-reactivity with cruciferous vegetables such as broccoli and cabbage, thus representing a crucial aspect of mustard's allergenic profile (43).

Such allergens can result in a range of symptoms, including hives, respiratory difficulties and anaphylaxis, particularly when cross-reactive proteins are present in other foods. The use of component-specific IgE testing is an effective method for identifying sensitization to Sin a 2, Sin a 3, and Sin a 4, which in turn facilitates an accurate diagnosis and dietary management plan. In cases where individuals are at high risk of severe reactions, it is essential to strictly avoid mustard and cross-reactive foods, while also carrying an epinephrine auto-injector (44).

Symptoms of a mustard allergy can include skin symptoms such as hives, swelling and itching, as well as respiratory problems such as difficulty breathing and asthma attacks (45, 46). Some people also experience food allergic reactions such as nausea, vomiting and abdominal pain. These symptoms can range in severity from mild to life-threatening. Bueno-Díaz *et al.* (47), in a study of albumin 2 S allergens, found that the main symptoms of hypersensitivity to this group of antigens are systemic reactions such as anaphylaxis and gastrointestinal syndrome. In addition, these antigens are available in many other products. Dreskin *et al.* (48) found these allergens in sesame, peanuts, and nuts. This is one of the reasons for the cross-reaction between these products. In addition, Achour *et al.* (49) reports that 2S albumin is present in sunflower oil, which significantly limits the diet of patients with this allergy. Lastly, according to Savvatianos *et al.* (50) this allergen was found in cashews and pistachios.

Mustard allergy is diagnosed by skin prick tests and blood tests for specific IgE antibodies. Skin prick tests can detect immediate allergic reactions to mustard, and a blood test helps to confirm the diagnosis and determine the degree of sensitization. In some cases, provocative tests may be required to clarify the diagnosis and assess the severity of the reaction (51). Treatment for a mustard allergy includes eliminating the spice from the diet and using medication to manage symptoms. Antihistamines can help manage skin and respiratory symptoms, and epinephrine may be needed for severe reactions such as anaphylactic shock (52, 53). It is also important to educate patients to recognise allergy symptoms and take precautions when eating outdoors.

Prevention of allergic reactions to mustard includes reading food labels carefully and avoiding foods containing this spice. People with severe allergies should always carry emergency equipment, such as epinephrine auto-injectors, and inform others of their allergies. This is especially important in public places and when visiting restaurants. In conclusion, mustard allergy is a significant problem that requires a careful approach to diagnosis and management. Careful diagnosis and effective treatment can sig-

nificantly improve the quality of life of patients, helping to avoid adverse reactions and minimize the risk of serious complications.

Other seasonings and their impact on allergic reactions

In addition to the above-mentioned spices, other popular spices such as coriander, basil, rosemary, sage and thyme can also cause allergic reactions. Although allergies to these seasonings are less common, they still pose a risk to sensitive people. One of the problems with spice allergies is the potential for cross-reactivity. This means that a person allergic to one spice may also react to other spices containing similar allergenic proteins. For example, people who are allergic to basil often react also to oregano and rosemary.

Coriander, also known as cilantro, can cause allergic reactions, especially in people with allergies to carrots and celery due to cross-reactivity. The main symptoms include itching, hives and, in some cases, anaphylactic shock (54). Coriander allergens include the proteins profilins and furanocoumarins, which can trigger an immune response in sensitive people (55).

Basil, used in Mediterranean cuisine, contains allergens similar to those of birch pollen, which can cause symptoms ranging from mild itching to serious respiratory problems. Cross-reactivity with oregano and rosemary means that people sensitive to basil should be careful with these spices (56).

Rosemary, popular in cooking for its aroma, may provoke skin rashes, itching, and even respiratory symptoms in sensitive individuals. People with rosemary allergy often react to sage and basil, which requires a cautious approach to their use (57).

Sage, often used for medicinal purposes and in cooking, contains allergenic proteins that can cause allergic reactions (58). Symptoms include skin manifestations, respiratory problems and, in rare cases, anaphylactic shock. Cross-reactivity with rosemary and basil is also common. Sage is a member of the Lamiaceae family, which means that people with sage allergy may also have cross-reactions to other herbs in the family, such as mint, oregano, basil and thyme (59). Thyme, popular in European cuisine, can cause allergic reactions in some people. Allergens can lead to skin rashes, itching and respiratory symptoms. People allergic to thyme should be careful when using other spices such as rosemary and sage (60).

Thus, spice allergy is a significant problem that requires attention from both healthcare professionals and food manufacturers. An integrated approach to the diagnosis, treatment and prevention of spice allergies can significantly improve the quality of life of sensitive people and prevent the development of serious complications.

The findings of this review underscore the significant clinical impact of spice allergies, which are frequently under-recognized despite their potential severity. The clinical presentation of spice allergies can range from mild cutaneous symptoms to severe anaphylactic reactions. This variability in symptoms highlights the necessity for heightened clinical awareness among healthcare professionals. The implementation of enhanced diagnostic techniques, such as more precise IgE tests, is of paramount importance for the accurate identification of patients at risk. A significant implication for public health is the cross-reactivity between spices and other plant-based allergens, which presents a challenge to allergy management and may result in accidental exposure in individuals with a sensitivity to these substances.

A notable deficiency in the extant research is the absence of epidemiological data concerning the prevalence of spice allergies across diverse populations and geographical regions. The majority of studies have concentrated on specific allergens, frequently within the context of isolated clinical scenarios, which has resulted in a fragmented understanding. Moreover, there is a paucity of research investigating the molecular mechanisms underlying spice allergies. Elucidation of these mechanisms could facilitate the development of targeted therapies and enhance diagnostic accuracy.

It is recommended that future researches aim to standardize diagnostic protocols, especially in cases of suspected cross-reactivity. It is imperative that large-scale epidemiological studies be conducted in order to accurately ascertain the prevalence of spice allergies and to gain insight into the regional variations that exist. Furthermore, molecular studies exploring the structural components of spice allergens could facilitate the development of allergen-specific immunotherapy, which may offer a potential pathway for long-term management. Such research would not only address existing knowledge gaps but also facilitate the development of dietary guidelines and public health interventions designed to mitigate the risks associated with spice allergies.

Conclusions

Allergies to spices are a significant problem for many people and can cause a wide range of symptoms, from mild skin rashes to serious respiratory problems and anaphylactic shock. Pepper, cumin, oregano and anise are examples of such spices, each of which contains specific allergens that can trigger an immune response in sensitive people. The main pepper allergens are PR-10 proteins, profilins and defensins, which can cause cross-allergic reactions with pollen and other plant allergens. Cumin may provoke strong allergic reactions in certain cases. Oregano contains Bet v 1 and profilins, which often lead to cross-allergies with other spices and plants. Anise allergy is less common but can also be a serious problem, especially in people with birch pollen allergy, due to cross-reactivity.

Clinical manifestations of spice allergy include hives, swelling of the lips and throat, difficulty breathing, nausea and vomiting. These symptoms can occur immediately after consuming the spice or after a while, making diagnosis difficult. The cross-reactivity between different spices and allergens also makes it difficult to diagnose and manage allergies. Skin prick tests and blood

tests for specific IgE antibodies are used to confirm the diagnosis. Treatment of spice allergy is aimed at avoiding triggers and symptomatic treatment, including the use of antihistamines and, if necessary, epinephrine to stop anaphylactic reactions.
Thus, spice allergy requires a careful approach to diagnosis and treatment to avoid adverse reactions and improve the quality of life of patients. Improved awareness among healthcare professionals and patients, as well as the development of new therapeutic approaches, can significantly improve allergy management and minimize the risk of serious complications.

Fundings

None.

Contributions

MZL: contributed entirely to the work.

Conflict of interests

The author declares that she has no conflict of interests.

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ORIGINAL ARTICLE

Eur Ann Allergy Clin Immunol Vol 58, N.2, 61-68, 2026

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Expression of IL-17RA in innate cells of patients with common variable immunodeficiency (CVID) and its clinical implications

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Key words

CVID; primary immunodeficiency; interleukin-17; inflammation; innate immunity.

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Doi
10.23822/EurAnnACI.1764-1489.326

Impact statement

Despite low circulating IL-17 levels, CVID patients appear to have heightened IL-17RA expression in innate immune cells. This altered IL-17 signaling may sustain a pro-inflammatory state, influenced by microbial colonization.

Summary

Background. *Common variable immunodeficiency (CVID) is a primary immunodeficiency disorder characterized by B-cell dysfunction and immunoglobulin production deficiency. Dysregulation of interleukin-17 (IL-17) and its receptor IL-17RA have been reported in various immune disorders. This study aimed to investigate the expression of IL-17RA in innate immune cells of CVID patients and its correlation with clinical manifestations.* ***Methods.*** *A cross-sectional study included 22 CVID patients and 14 age- and sex-matched healthy controls. IL-17RA expression was assessed in various immune cell subsets using flow cytometry. Demographic and clinical data were collected, and statistical analysis was performed.* ***Results.*** *CVID patients had elevated IL-17RA expression in neutrophils, non-classical monocytes, and dendritic cells compared to healthy controls. Patients with a history of intestinal microbial colonization, particularly with Campylobacter jejuni and Giardia intestinalis, showed significantly higher IL-17RA expression in innate cells. Elevated IL-17RA expression in monocytes and dendritic cells also correlated with higher fecal calprotectin levels in CVID patients, regardless of microbial colonization.* ***Conclusions.*** *The study suggests that IL-17RA expression in innate cells may be elevated, potentially indicating altered IL-17 signaling. This heightened IL-17RA expression could contribute to a persistent pro-inflammatory state, possibly due to microbial translocation or other inflammatory factors. The association of IL-17RA expression with gastrointestinal microbial colonization and its correlation with fecal calprotectin underscores the complexity of IL-17RA's role in CVID pathophysiology.*

Introduction

Common variable immunodeficiency (CVID) is the most common symptomatic inborn error of immunity (1). It is mainly characterized by failure of B-cell differentiation and decreased production of immunoglobulins (IgS). This leads to a predominantly humoral immunodeficiency, despite also being associated with cell-mediated deficiencies (2). Aside from B-cell dysfunction, other immunological abnormalities, such as T-cell dysfunction, monocyte/macrophage hyperactivity and abnormal cytokine production, with subsequent inflammatory dysregulation, are observed in many patients (3). This wide presentation of immu-

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nological defects reflects CVID's heterogeneous genetic abnormalities and it leads to diverse clinical manifestations (recurrent sinopulmonary infections, autoimmune disorders, granulomatous diseases, enhanced risk of malignancy, and impaired antibody response) (4).

The interleukin-17 (IL-17) family comprises a group of six pro-inflammatory cytokines (from the main cytokine IL-17A, also known as IL-17, through IL-17F) produced mainly by Th17 lymphocytes, but also by CD8+ T-cells, γδ T-cells, and various innate immune cell populations (5-7). IL-17 signals through the IL-17 receptor A (IL-17RA, CD217) and IL-17RC subunits. IL-17F, the most closely related family member, also binds this receptor complex (8). Whereas IL-17RA is ubiquitously expressed (with particularly high expression by innate immune cells such as macrophages and dendritic cells), lower IL-17RC expression limits IL-17 signaling in non-hematopoietic epithelial and mesenchymal cells (9). The physiologic expression of IL-17 has a significant impact in innate immunity, playing a role in responses against extracellular bacteria, fungi and parasites by directly recruiting monocytes and indirectly influencing neutrophils, mainly through interaction with epithelial cells (6, 10-12). It is also important for the barrier function of the skin and of the gut, by maintaining the tight junctions of the intestinal epithelium, stimulating tissue regeneration and upregulating antimicrobial proteins, such as β-defensins and calprotectin to control infections (13). By contrast, IL-17 chronic overactivity may elicit pathological responses, with Th17 cells having a major role in both cancer and autoimmune diseases (*e.g.*, psoriasis, rheumatoid arthritis or autoimmune encephalitis) (14).

Defects in the IL-17 pathway have been described for inborn errors of immunity (15). Studies in patients with CVID have demonstrated a severe reduction of circulating Th17 and innate lymphoid cells, with a similar trend for serum levels of IL-17 (3, 16). No relation was found between this decrease and autoimmune disorders, which occur frequently in CVID patients (3, 16, 17). However, the expression of IL-17RA, a receptor that is crucial for IL-17 signaling and innate immunity regulation, is largely unknown in the innate immune cells of CVID patients. Therefore, we examined the expression of IL-17RA in the circulating innate immune cells of CVID patients and looked to determine how it correlated with the clinical manifestations of the disease.

Materials and methods

Study design and subject recruitment

A cross-sectional study was conducted at the Allergy and Clinical Immunology Unit of Coimbra University Hospital, Portugal. A total of 22 patients with a clinical diagnosis of CVID, according to the diagnostic criteria of the European Society for Immunodeficiencies (ESID) Registry Working Party, were consecutively enrolled from 2018 to 2022 and results were compared with 14 age and sex-matched healthy controls (absence of infectious disease in the previous three months, as well as neoplastic, autoimmune, or lymphoproliferative disease) (18). This project was approved by the local ethics committee review board. Informed consent for obtaining blood samples for functional assays were taken in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to participation.

IL-17RA expression analysis

The expression of IL-17RA (CD217 antigen) was assessed by flow cytometry in myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), three subsets of monocytes (classical, intermediate and non-classical), and neutrophils. The following antibodies (and respective clones) were used: CD16 FITC (CLB/FcGRAN1), CD278 PE (C398.4A), CD3 PerCPCy5.5 (SK7), HLA-DR PE-Cy7 (L243), CD8 APC-H7 (SK1), CD45 V500c (2D1), CD217 APC (W15177 A), and CD14 APC-H7 (M Ø P9). Gating strategy for monocytes and dendritic cells is represented in **figure 1**.

Data collection

Demographic and clinical data were collected from CVID patients through review of clinical records. Demographic variables included were sex, age at CVID diagnosis and age at cytometry flow analysis. Clinical variables included the following: phenotypes (updated 2012 criteria proposed by Chapel *et al.* – autoimmune cytopenia, polyclonal lymphocytic infiltration and unexplained enteropathy), history of malignancy, asthma/COPD, chronic rhinosinusitis, pneumonia, chronic diarrhea, hepatomegaly, splenomegaly and/or bronchiectasis, infectious colonization and isolated microorganisms, IgG through levels' measurements and blood cell count, IgG and maximum fecal calprotectin measurements up to the time of cytometry analysis (19). Infectious colonization was defined as the analysis of microorganisms (parasites, bacteria and fungi) in the respiratory and gastrointestinal tracts – particularly, sputum and stool cultures, as well as antigen measurement in stool samples. Patients with identification of microorganisms in these samples were defined as colonized. Collected information was then analyzed with the flow cytometry data.

Statistical analysis

All statistical analyses were done using STATA software (version 16.1, StataCorp LLC, Texas, USA). Categorical data was described as a proportion and continuous data either as mean or median, depending on normality. P-values of < 0.05 for correlations were considered statistically significant. Chi-squared or Exact Fisher tests were used for associations between categorical variables. T-test or Mann-Whitney U statistical analysis was also conducted, with the test choice depending on observation of normality. After univariate analysis, a multivariate linear regression model was built in order to assess the impact of intestinal tract

colonization in the association between mean fecal calprotectin levels and CD217 cell expression.

Results

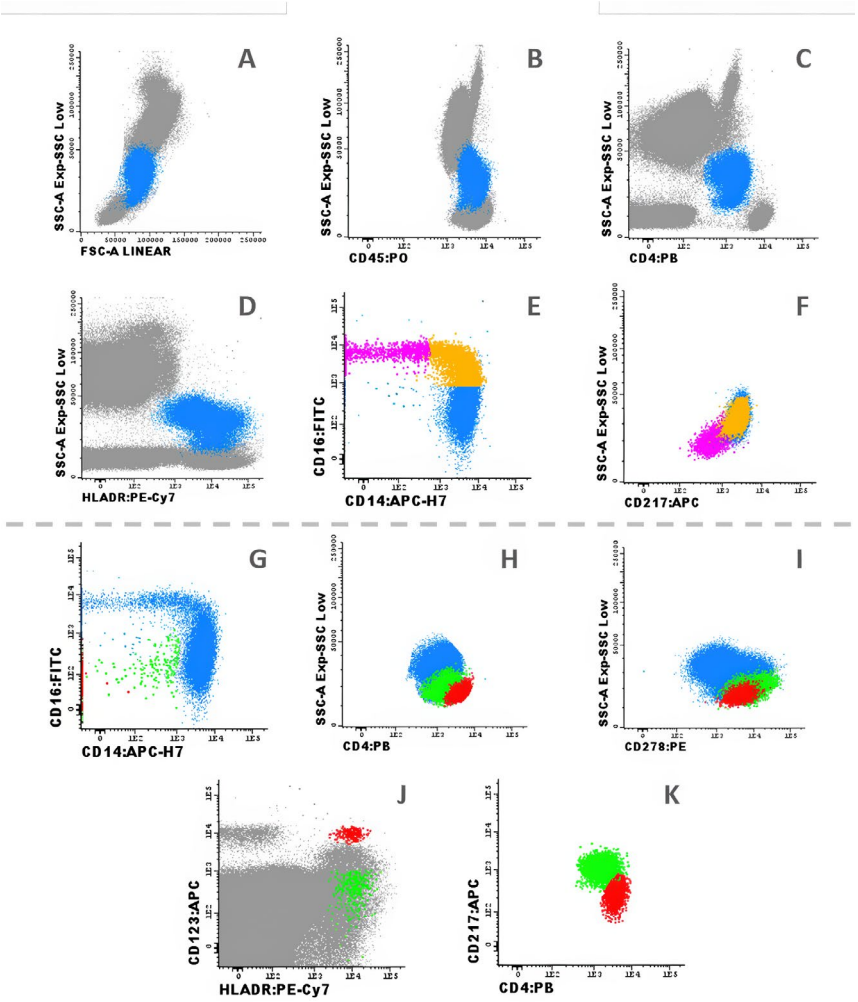
Demographic characteristics

CVID patients (n = 22) had a median age at the time of flow cytometry analysis of 48 years (IQR 44-56). Ten patients were male (45.5%). The median age of CVID diagnosis was 35 years (*IQR 28-45*). Healthy controls (n=14) were age- and sex-matched and, therefore, not significantly different from patients (**table I**).

Clinical characteristics of CVID patients

Regarding Chapel phenotypes, 11 (50%) patients presented with autoimmune cytopenia, 10 (45.5%) with polyclonal lymphocytic infiltration and 6 (27.3%) with chronic enteropathy (**table II**). Four patients (18.2%) had no disease-related complications, and 7 (31.8%) presented with more than one phenotype. Imaging stud-

Figure 1 *- Representative dot plots illustrating the identification of monocytes* ***(A-F)*** *and dendritic cells* ***(G-K)*** *subtypes, in peripheral blood samples, using a combination of eight-color mouse anti-human antibodies.*



(E) Monocytes: classical monocytes in blue (based on the positive expression of CD14 and negative expression of CD16), intermediate monocytes in yellow (based on the positive expression of both CD14 and CD16) and non-classical monocytes in pink (based on the positive expression of CD16 and dim/negative expression of CD14); **(J, K)** Dendritic cells: defined by HLA-DR and CD4 expression in the absence of CD14 and CD16. Plasmacytoid dendritic cells exhibit a higher expression of CD4 when compared with myeloid dendritic cells, as well as a dim expression of CD217 (confirmed with the specific combination of CD123 and HLA-DR that allows for a better definition of these two subsets): plasmacytoid dendritic cells (pDC) in red and myeloid dendritic cells (mDC) in green.

Table I *- Comparison between both groups: demographic characteristics, cell counts and CD217 expression.*

Variables	CVID (n = 22)	Controls (n = 14)	P-value
Median age at measurement (IQR), years	48 (44-56)	49 (40-58)	0.602
Male gender, %	45.5	42.9	0.878
Leukocyte count (IQR), n x 10^6/L	6,850 (4,600-10,100)	6,200 (4,800-7,000)	0.390
Lymphocyte count (IQR), n x 10^6/L	1,521 (1,029-2,177)	2,064 (1,688-2,359)	0.049*
Median overall proportion of cell subtypes (IQR), %			
Lymphocytes	24.2 (16.0-30.0)	35.0 (32.9-37.7)	< 0.001*
B-Cells	5.1 (4.0-8.3)	21.2 (15.0-29.8)	< 0.001*
T-Cells	73.1 (64.0-88.2)	71.8 (63.2-78.1)	0.545
CD4+	60.8 (48.4-64.2)	64.8 (60.3-72.6)	0.014*
CD8+	35.4 (31.2-42.0)	26.8 (22.7-32.8)	0.008*
Gamma-delta	3.2 (1.7-5.6)	3.0 (2.3-5.2)	0.978
Triple-negative	1.7 (1.4-3.6)	1.3 (1.0-1.5)	0.039*
Neutrophils	63.2 (56.0-71.0)	52.4 (47.2-57.9)	0.003*
Monocytes	6.8 (5.7-9.3)	8.4 (7.3-9.6)	0.058
Classic	83.7 (79.6-89.7)	79.0 (73.2-81.1)	0.014*
Intermediate	7.7 (3.8-12.9)	10.8 (9.0-13.4)	0.162
Non-classic	5.0 (3.6-8.6)	11.2 (9.2-12.9)	< 0.001*
Myeloid dendritic cells	0.14 (0.10-0.20)	0.26 (0.23-0.32)	< 0.001*
Plasmacytoid dendritic cells	0.04 (0.02-0.10)	0.14 (0.09-0.20)	< 0.001*
CD217 cell expression by median MFI (IQR), units			
Neutrophils	1,908 (1,634-2,143)	1,474 (1,182-1,807)	0.003*
Classic monocytes	2,295 (1,943-2,747)	2,134 (1,714-2,368)	0.289
Intermediate monocytes	1,956 (1,629-2,313)	2,033 (1,440-2,304)	0.803
Non-classic monocytes	1,132 (810-1,345)	864 (585-995)	0.012*
Myeloid dendritic cells	1,438 (1,309-1,828)	1,135 (987-1,282)	< 0.001*
Plasmacytoid dendritic cells	509 (409-633)	344 (213-433)	< 0.001*

IQR: interquartile range; MFI: mean fluorescence intensity; *statistically significant ($p < 0.05$).

ies showed hepatomegaly in 5 (22.7%) patients, splenomegaly in 9 (40.9%) and bronchiectasis in 14 (63.6%). Asthma/COPD was present in 7 patients and chronic rhinosinusitis in 18 (81.8%). A past history of pneumonia was registered in 13 patients (59.1%), chronic diarrhea in 12 (54.5%) and malignancy in 3 (13.6% with gastric, colorectal and thyroid cancer).

Fourteen patients had a history of microbial colonization: 11 in the respiratory airways and the same number in the gastrointestinal tract. The microorganisms isolated in sputum were *H. influenzae* (n = 10), *P. aeruginosa* (n = 2), *M. catarrhalis* (n = 2), *S. pneumoniae* (n = 2), *S. aureus* (n = 1), and *Aspergillus niger* (n = 1). In the gastrointestinal tract, the isolated bacteria were *H. pylori* (n = 10), *C. jejuni* (n = 7) and *S. enterica* (n = 1), as well as the parasite *Giardia intestinalis* in 4 patients.

Relative blood cell count

In peripheral blood, CVID patients had no significant differences in leucocyte cell absolute count in comparison to controls (median 6,850 *vs* 6,200 × 10^3/L, p = 0.390), but lymphocyte count was lower (median 1,521 *vs* 2 064 × 10^3/L, p = 0.048). Among the lymphocyte subgroup, the proportion of B-cells was significantly lower in CVID patients (5.1 *vs* 21.2%, p < 0.001), with no difference between T-cells; CVID patients also had a lower proportion of CD4+ T-cells when compared to controls (60.8 *vs* 64.8%, p

= 0.014), which contrasted with higher CD8+ (35.4 *vs* 26.8%, p = 0.007) and triple-negatives (CD4-CD8-γδ- T-cells – 1.7 *vs* 1.3%, p = 0.039), with no significant differences in γδ T-cells. Regarding innate cells, the overall proportion of mDCs (0.15 *vs* 0.26%, p < 0.001), pDCs (0.04 *vs* 0.14%, p < 0.001), and circulating non-classical monocytes (5.0 *vs* 11.2%, p < 0.001) was significantly lower in CVID patients. In contrast, there were significantly higher numbers of neutrophils (63.2 *vs* 52.4%, p = 0.003) and classical monocytes (83.7 *vs* 79.0%, p = 0.014).

CD217 expression in innate cells, based on the Mean Fluorescent Intensity (MFI)

Overall, CD217 expression was significantly higher in most innate cell types of CVID patients compared to controls (**table I**): neutrophils (1,908 *vs* 1,474, p = 0.003), non-classical monocytes (1,132 *vs* 864, p = 0.012), mDCs (1,438 *vs* 1,135, p < 0.001) and pDCs (509 *vs* 344, p < 0.001). No significantly different expression of CD217 was found in classical and intermediate monocytes between the two groups.

We then compared how CD217 expression was related to CVID clinical manifestations. Patients with a history of intestinal colonization had a significantly higher expression of this receptor in comparison with non-colonized CVID patients. No differences in CD217 expression were observed in relation to other demographic or clinical characteristics, such as IgG levels, respiratory tract colonization, asthma/COPD, chronic rhinosinusitis, chronic diarrhea, malignancy, Chapel phenotypes or particular imaging findings. Taking into consideration the heterogeneous manifestations of CVID, we divided CVID patients in two major clinical groups: 1) patients with immunodysregulatory complications (autoimmunity, malignancy, enteropathy and/or lymphoid hyperplasia – n = 16); and 2) patients mainly with infections manifestations (n = 6). No significant differences in CD217 expression of innate cells were observed between the groups.

It should be noted that, even though CVID patients with intestinal microbial colonization had higher expression of CD217 in innate cells, patients without microbial colonization still expressed more CD217 when compared to healthy controls.

Specifically, a higher CD217 expression was observed in the innate cells of CVID patients with a history of gastrointestinal colonization by *Campylobacter jejuni* and *Giardia intestinalis* (**figure 2**). More specifically, CD217 expression in all monocyte cell subtypes – classical (controls: 2,112 *vs* CVID patients: 2,845, p = 0.007), intermediate (1,658 *vs* 2,524, p = 0.012) and non-classical (1,000 *vs* 1,460, p = 0.026) – and in both DC types – mDCs (1,410 *vs* 1,858, p = 0.019) and pDCs (476 *vs* 622, p = 0.056) – was significantly higher in the 7 patients with a history of *Campylobacter jejuni* colonization. Although only 4 patients had been colonized with *Giardia intestinalis*, this sub-group also achieved a statistically significant higher CD217 expression in non-classical monocytes (1,714 *vs* 1,084, p = 0.039), in comparison to non-colonized patients.

CD217 expression in mDCs (r = 0.230, p = 0.042) and classical monocytes (r = 0.627, p = 0.031) was associated with higher mean fecal calprotectin levels in CVID patients, even after controlling for gastrointestinal colonization history (**table III**).

***Table II** - Clinical characterization of CVID patients (n = 22).*

Clinical variables	Yes	No	Unknown
History of microbial colonization, n (%)			
Total	14 (63.6)	7 (31.8)	1 (4.6)
Respiratory agents	11* (50.0)	9 (40.9)	2 (9.1)
Gastrointestinal agents	11** (50.0)	11 (50.0)	0 (0.0)
Chapel phenotype characteristics, n (%)			
Autoimmunity	11 (50.0)	11 (50.0)	0 (0.0)
Unexplained enteropathy	6 (27.3)	16 (72.7)	0 (0.0)
Polyclonal lymphoproliferation	10 (45.5)	12 (54.5)	0 (0.0)
Other clinical characteristics, n (%)			
Hepatomegaly	5 (22.7)	17 (77.3)	0 (0.0)
Splenomegaly	9 (40.9)	13 (59.1)	0 (0.0)
Bronchiectasis	14 (63.6)	6 (27.3)	2 (9.1)
Asthma/COPD	7 (31.8)	4 (18.2)	0 (0.0)
Chronic rhinosinusitis	13 (59.1)	9 (40.9)	0 (0.0)
Chronic diarrhea	12 (54.5)	10 (45.5)	0 (0.0)
History of pneumonia	12 (54.5)	10 (45.5)	0 (0.0)
History of malignancy	3 (13.6)	19 (86.4)	0 (0.0)
Laboratory variables, median (IQR)			
Pre-treatment IgG levels, g/L	2.4 (0.8-4.1)		
Current IgG levels, g/L	8.0 (7.1-9.9)		
Fecal calprotectin levels, µg/g	330 (230-781)		

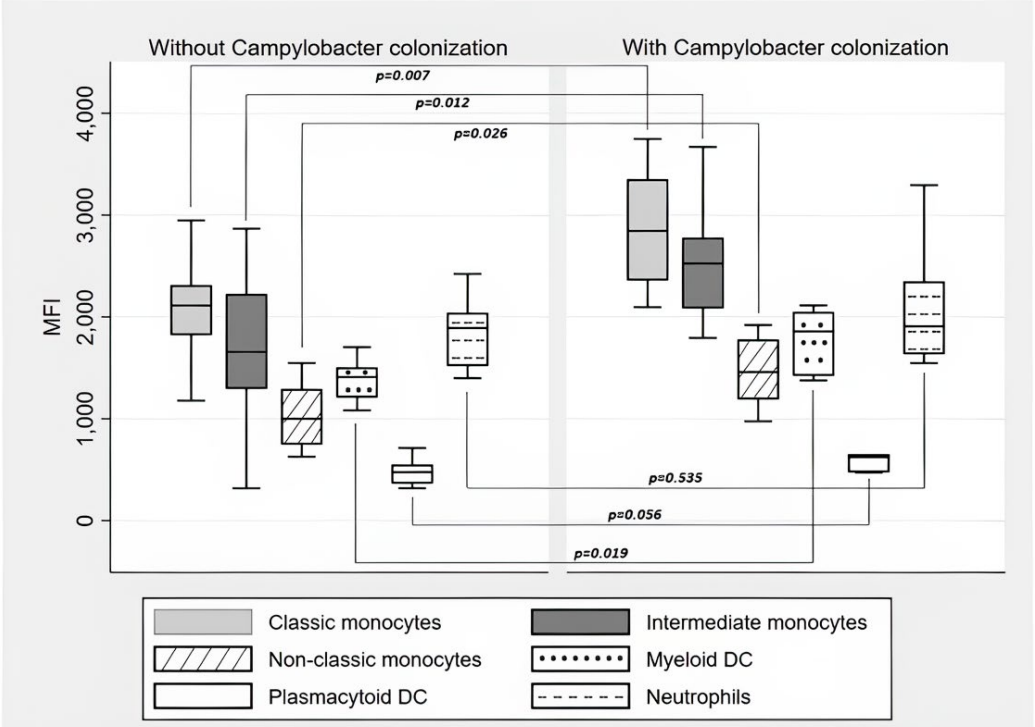
IQR: interquartile range; IgG: Immunoglobulin G. **H. influenzae* = 10; *P. aeruginosa* = 2; *M. catarrhalis* = 2; *S. pneumoniae* = 2; *S. aureus* = 1; *Aspergillus niger* = 1. ***H. pylori* = 10; *C. jejuni* = 7; *S. enterica* = 1; *Giardia intestinalis* = 4.

Discussion and conclusions

In summary, our study reveals a significant increase in the expression IL-17RA in innate cells of CVID patients such as neutrophils, non-classical monocytes, and dendritic cells. Moreover, its heightened expression in patients with a history of intestinal colonization suggests a potential association with persistent pro-inflammatory states and microbial infections, hinting at a role in gut inflammation.

Previous published studies reported a decrease in both circulating Th17 cells and IL-17 levels in patients with CVID (15).

Figure 2 *- CD217 expression in innate cells: comparison according to Campylobacter and Giardia colonization.*



Cell subtype	*Campylobacter jejuni*		*p-value*	*Giardia intestinalis*		*p-value*
	Colonized (n=14)	Non-colonized (n=7)		Colonized (n=4)	Non-colonized (n=17)	
CD217 cell expression by median MFI (IQR), units						
Neutrophils	1908 (1634-2350)	1891 (1518-2042)	*0.535*	2048 (1610-2822)	1908 (1634-2042)	*0.635*
Classic monocytes	2845 (2358-3353)	2112 (1820-2314)	*0.007**	2724 (1637-3550)	2295 (1943-2474)	*0.635*
Intermediate monocytes	2524 (2085-2780)	1658 (1293-2225)	*0.012**	2430 (1606-3223)	1794 (1629-2308)	*0.462*
Non-classic monocytes	1460 (1189-1779)	1000 (745-1293)	*0.026**	1714 (1291-1850)	1084 (773-1281)	*0.039**
Myeloid dendritic cells	1858 (1421-2051)	1410 (1207-1506)	*0.019**	2051 (1376-2114)	1429 (1299-1704)	*0.146*
Plasmacytoid dendritic cells	622 (473-654)	476 (363-551)	*0.056*	553 (472-654)	489 (389-622)	*0.546*

IQR - interquartile range; MFI - mean fluorescence intensity; *- statistically significant ($p<0.050$)

However, our study shows that the expression of main receptor IL-17RA may follow an opposite trend, with a statistically significant increase in most innate cells (neutrophils), non-classical monocytes and dendritic cells. This is an important finding, as previously described, because increases in Th17 and IL-17 might not translate into improved IL-17-mediated innate responses in these patients due to lack of IL-17RA signaling.

Patients with CVID are known to present with an altered cytokine profile that is consistent with a persistent activation of monocytic and granulocytic cell lineages (20). This immune activation has been partially explained by chronic microbial translocation, mainly bacterial and fungal, in the respiratory and gastrointestinal tracts of CVID patients (21). IL-17 has a major role in host defense against microbial pathogens by activating IL-17RA and inducing a pro-inflammatory cascade that activates and recruits neutrophils and monocytes for pathogen control. Additionally, the IL-17/IL-17R signaling pathway appears to be stimulated simultaneously, with the blockade of inhibitors such as PI3K or TRAF3 leading to elevated expression of IL-17RA and consequently strengthening IL-17 signaling (22, 23).

Therefore, we hypothesized that the increased expression of IL-17RA in the innate cells of CVID patients is associated with a persistent pro-inflammatory state that is observed in these patients. Alternatively, low circulating levels of Il-17 may lead to increased expression of IL-17RA through some unidentified feedback regulation mechanism.

Particularly high levels of IL-17RA were found in patients with a history of intestinal colonization with *Campylobacter* or *Giardia*. IL-17 has an intense activity in intestinal epithelial regeneration and also plays essential roles in host defense against microbial pathogens, including respiratory tract bacteria such as *K. pneumoniae*, as well as gastrointestinal bacteria such as *S. enter-*

Table III *- Linear regression analysis of CD217 cell expression, with calprotectin and gastrointestinal colonization as co-variates.*

Variable	Regression coefficient	Standard-error	P-value
Neutrophils - CD217 expression by MFI, units			
Fecal calprotectin	0.185	0.144	0.228
Gastrointestinal colonization	-148.0	199.2	0.475
Classic monocytes - CD217 expression by MFI, units			
Fecal calprotectin	0.627	0.249	0.031*
Gastrointestinal colonization	-142.4	345.4	0.689
Intermediate monocytes - CD217 expression by MFI, units			
Fecal calprotectin	0.251	0.243	0.326
Gastrointestinal colonization	102.1	336.7	0.768
Non-classic monocytes - CD217 expression by MFI, units			
Fecal calprotectin	0.192	0.152	0.240
Gastrointestinal colonization	155.7	222.2	0.501
Myeloid DC - CD217 expression by MFI, units			
Fecal calprotectin	0.230	0.097	0.042*
Gastrointestinal colonization	167.5	141.2	0.266
Plasmacytoid DC - CD217 expression by MFI, units			
Fecal calprotectin	-0.078	0.091	0.416
Gastrointestinal colonization	259.1	133.2	0.084

DC: dendritic cells; MFI: mean fluorescence intensity; *statistically significant ($p < 0.05$).

ica, fungus like *C. albicans,* and parasites like *Trypanosoma* (24-26). However, we found no studies correlating IL-17 and microbial colonization in CVID, and the specific pattern of pathogen infections in our cohort could explain these results. It should be noted that this study's sample size limits further interpretations. Applying these measurements in a larger cohort could not only help confirming our hypothesis, but also include other pathogens that are commonly found in CVID patients, such as fungi. Even though levels of IL-17RA were significantly higher in CVID patients with gastrointestinal microbial colonization, the subgroup of patients without microbial colonization still expressed higher IL-17RA levels in innate cells when compared with healthy controls. Besides mucosal barrier protection against microorganisms, IL-17 also has an important function as a mediator in autoimmunity and cancer. About 20% of CVID patients present with autoimmune diseases or other auto-inflammatory manifestations such as lymphoid hyperplasia, granulomatous infiltrations, and "inflammatory bowel disease-like" colitis (14). Therefore, we cannot exclude that other pro-inflammatory factors aside from microbial colonization could lead to increased IL-17RA expression.

Th17 cells play a major role in the development of many autoimmune diseases. Thus, considering the potential impact of pro-inflammation in IL-17RA expression, we compared CVID patients with and without a history of autoimmunity (particularly cytopenia), but no significant differences were found. Interestingly, Il-17RA regulation could be significantly regulated by bowel auto-inflammatory processes (receptor expression in innate immune cells from patients with CVID correlated significantly with higher fecal calprotectin levels, independently of gastrointestinal colonization). Calprotectin is produced mainly by innate immune cells, and its pathophysiological relationship with IL-17RA requires further in-depth studies. It means there could be a dysregulation of the inflammatory activity in the gut promoted by IL-17; alternatively, IL-17 could also be having a regulatory effect, by promoting mucosal barrier protection in response to inflammatory or infectious mechanisms (27).

This study has some limitations to be taken in consideration. As previously noted, statistical analysis is limited by the small cohort, and there is an absence of cytokine serum measurements, including Il-17, that may hinder our mechanistic understanding of IL-17RA expression. Furthermore, both the distinct immune phenotypes of CVID patients regarding B and T-cell profiles and the heterogeneous genetic background can have a potential correlation with clinical manifestations and complications. As such, despite our small number of CVID patients, this study could have benefited from the inclusion of genetic characterization, as well as immune profiling according to the Euroflow and/or EUROclass phenotypes (4, 28, 29).

The expression of IL-17RA and IL-17 signaling in the innate immune system of CVID patients might have important implications in infectious and non-infectious inflammatory manifestations of the disease. A better understanding of these observations might be achieved in larger cohorts of patients with detailed clinical, immunological and genetic characterization, paired with the measurement of IL-17 serum levels.

Fundings

None.

Contributions

PBA: conceptualization, formal analysis, writing – original draft. HP, JC, IN: data curation. ATB: writing – review & editing. EF, FR: conceptualization, writing – review & editing. AP: supervision, conceptualization, writing – original draft, writing – review & editing.

Conflict of interests

The authors declare that they have conflict of interests.

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ORIGINAL ARTICLE

Eur Ann Allergy Clin Immunol Vol 58, N.2, 69-76, 2026

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Comparison of the characteristics of patients with chronic urticaria receiving standard- or high-dose omalizumab

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Key words

Urticaria; omalizumab updosing; D-dimer; anti-TPO positivity; low IgE.

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Doi
10.23822/EurAnnACI.1764-1489.380

Impact statement

D-dimer level seems to be a predictor for omalizumab updosing. IgG-anti-TPO positivity and low IgE do not predict the need for dose escalation.

Summary

Background. *In patients whose chronic urticaria (CU) cannot be controlled with omalizumab 300 mg and antihistamines, the dose can be increased up to 600 mg. The study aimed to compare the clinical characteristics of patients receiving 300 mg versus higher doses of omalizumab, and to evaluate baseline predictors for updosing.* ***Methods.*** *A total of 159 patients who have been followed up at a tertiary care allergy center and received omalizumab for at least 12 months were included. The clinical characteristics of those who received the standard-dose omalizumab (Group 1) were compared to the ones who received therapy over the standard dose (Group 2).* ***Results.*** *A total of 139 (87%) were in Group 1, and 20 (13%) were in Group 2. CU duration at baseline was shorter in Group 2. Chronic inducible urticaria was present in 2%, and 40% of the patients in Group 1 and 2, respectively. Elevated D-dimer level was associated with high-dose omalizumab use ($p < 0.001$). Area under the curve in the ROC analysis was 0.812 and the cutoff value of D-dimer level was 0.46 mg/dl ($p = 0.001$, sensitivity and specificity 67%, and 84%, respectively). The anti-TPO positivity was higher in patients with low IgE (31% vs 8%, $p = 0.008$).* ***Conclusions.*** *Nearly one in every ten patients required higher doses of omalizumab therapy. D-dimer level seems to be a predictor for omalizumab updosing and unresponsiveness to standard dose. IgG-anti-TPO positivity and low IgE do not predict the need for dose escalation; however, this result should be strengthened with a larger number of patients.*

Introduction

Chronic urticaria (CU) is a disease that persists for more than six weeks, with itchy and edematous papules/plaques, and may be associated with angioedema due to deep dermis or subcutis involvement, or with the development of both (1). The prevalence of CU varies between 0.5-1% in the general population (2). Identifiable triggering factors are stress, trauma, medications, food additives, temperature changes, pressure, surgery, hormones, physical exercise, autoimmunity, and infections (3, 4). While it is primarily recommended to move away from detectable triggers, some patients need to take regular treatment. While modern second-generation antihistamines (sgAHs) constitute the main drugs in the first step of the treatment algorithm, it is recommended to initiate omalizumab as a second-line treatment in patients who do not respond to high-dose sgAHs (5, 6). Undoubtedly, the use of omalizumab in different doses is effective in remission and the maintenance of this remission (7). However, with the standard dose of 300 mg, adequate control may not be achieved in approximately 30% of patients treated for six months. Several studies have found that a significant number of patients with chronic spontaneous urticaria (CSU) can be effectively treated with higher doses of omalizumab who tend to have higher body mass index (BMI), older age, and low immunoglobulin E (IgE) levels (8, 9). Predictable risk factors to clinically distinguish patients requiring high-dose omalizumab from those receiving standard-dose omalizumab have still not been clearly identified. The study aimed to compare the clinical characteristics of patients receiving standard- or high-dose omalizumab and to disclose the likely predictors to be in the high-dose group.

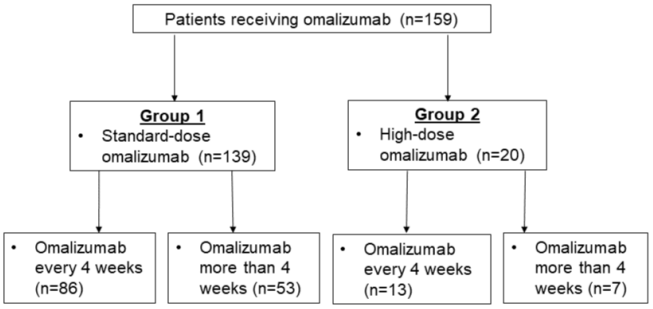
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Materials and methods

Study design and participants

The study was conducted as a prospective, single-center, cross-sectional survey. This observational real-life study included a total of 159 CU patients receiving omalizumab from September 1, 2014, to July 31, 2023 at a tertiary care allergy clinic. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki, revised in 2013, and was approved by Hacettepe University Ethics Committee (2023/11-04). In accordance with the recommendations of Zuberbier *et al.*, patients with compatible clinical histories were diagnosed with chronic spontaneous urticaria and chronic inducible urticaria (10). As of August 30, 2014, omalizumab was approved for use in antihistamine-resistant urticaria in Turkey and started to be used in our clinic. Inclusion criteria were provision of written informed consent, age ≥ 18 years, and use of omalizumab for at least 12 months. One (0.6%) patient diagnosed with urticarial vasculitis and treated with 600 mg of omalizumab was excluded from analysis. Study groups are shown in **figure 1**. Individuals receiving standard- or high-dose omalizumab were defined as Groups 1 and 2, respectively.

Figure 1 - *The flow chart of the study population.*



Data collection and laboratory measurements

The patients' pre-omalizumab or baseline laboratory values were recorded from the hospital database retrospectively. During regular hospital visits or omalizumab prescriptions and/or injections, informed consent was obtained through face-to-face interviews, and a questionnaire was subsequently used to prospectively document their current treatments, treatment durations, times of treatment changes, attacks, disease control, and medication adherence. In addition to the demographic characteristics of the patients, the survey form included the questions about the disease duration, the presence of angioedema, allergic diseases, urticaria exacerbations requiring systemic corticosteroids in the last year, admissions to the emergency room due to urticaria attacks, omalizumab doses and intervals, comorbidities, medications, urticaria control, baseline blood eosinophil and basophil counts, antinuclear antibody (ANA), IgG-anti-thyroid autoantibody (IgG-anti-TPO), specific immunoglobulin E (IgE) level (for the phadiatop, food mix, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat, dog, *Aspergillus fumigatus*, alternaria) or skin prick test (including *Phleum pratense*, *Artemisia vulgaris*, *Parietaria officinalis*, *Corylus avellena*, *Betula verrucosa*, *Olea europaea*, *D. pteronyssinus*, cat, dog, *Alternaria alternata*, *Claudosporium herbarum*, *Aspergillus fumigatus*, latex, blatella, acarus siro, positive control, negative control, corn, hazelnuts, peanuts, walnuts, almonds, chicken, egg white, egg yolk, orange, lemon, banana, peach, strawberry, cherry, tomato, bean, carrot, pea, crab, shrimp, mussel) results, pre-treatment C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), D-dimer level, total IgE level, urticaria control test (UCT), and medication adherence report scale were applied to all patients during their visits. Serum total IgE was measured by a chemiluminescent immunoassay (ImmunoCAP; Thermo Fisher Scientific, Sweden), and the cutoff value of low IgE level was < 43 IU/mL (11). A cut-off of less than 50 $\times 10^6/mm^3$ was accepted as eosinopenia which was the most frequently used cutoff in the literature (12-14). Basopenia was accepted as less than $10 \times 10^6/mm^3$, as previously described (14, 15). The cutoff value for elevated ESR was calculated with (age/2) for men, and with [(age+10)/2)] mm/h for women (16, 17). D-dimer level was measured by enzyme-linked immunosorbent assay, and the reference range was 0-0.55 mg/L.

Assessments of the treatment responses and compliances

Disease control within the last month was determined by the UCT. The UCT is an easy-to-administer, self-reported questionnaire assessing disease control in patients with CU, and it provides a final score between 0 and 16. Those with scores < 12 points and an increase of < 3 compared to baseline are accepted as uncontrolled or non-responder, and those ≥ 12 points and an increase of ≥ 3 compared to baseline are accepted as controlled or responder (18). Medication adherence report scale (MARS) was also performed. The total score of the scale is 25 points. As the total score increases, the compliance stands for better. MARS is validated in Turkish version by Sen *et al.* (19).

Follow-up during omalizumab treatment

As second-line therapy standard-dose omalizumab was initiated to patients who were non-responders to high-dose sgAHs, considering the EAACI/GA²LEN/EuroGuiDerm/APAACI guidelines (20). If the urticaria was still uncontrolled after at least four months of 300 mg of omalizumab, the dose was increased to 450 mg. In patients whose urticaria was still uncontrolled for at least additional three months of 450 mg of omalizumab, the dose was increased to 600 mg as recommended by Kocaturk *et al.* (21). If urticaria was under control for at least six months, the patient was considered for either decreasing the dose or extension of the injec-

tion interval by one week after every injection. However, during the enrollment process, it was observed that some patients were not taking regular antihistamines, which resulted in inadequate urticaria control. It was decided to follow up with standard-dose omalizumab therapy while adding regular antihistamines to the treatment regimen. Also, individuals whose urticaria could not be controlled with high-dose omalizumab were investigated for immunodeficiency, vasculitis, and other systemic diseases, which could have been among the differential diagnoses (1). One of our patients was diagnosed with common variable immunodeficiency (CVID) and initiated on intravenous immunoglobulin (IVIG) therapy. Eight months after starting IVIG, the patient's urticaria was completely controlled, and the omalizumab dose was reduced to 450 mg, administered once every four weeks.

Statistical analysis

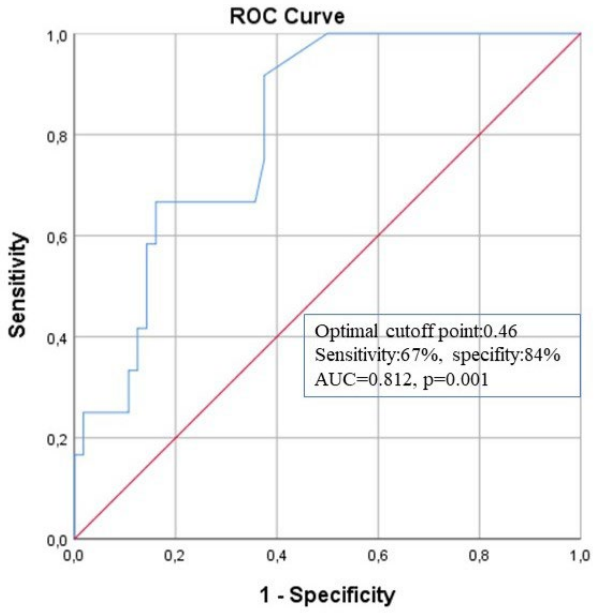
Data were analyzed using SPSS vn. 23.0 software. Descriptive data were presented as numbers (n) and percentages (%). Numerical variables showing normal distribution were stated as mean± standard deviation values, and otherwise as median and interquartile range (IQR) values. The Chi-Square or Fisher Exact test for categorical variables and the Mann-Whitney U or the Kruskal Wallis test for continuous variables were used. The independent samples t-test was applied to comparisons of parametric variables between two groups. Spearman correlation coefficient was used for non-normally distributed parameters. The possible factors identified with univariate analyses were further entered into the cox regression analysis, with backward selection according to the $p \leq 0.20$, to determine independent predictors of high-dose omalizumab use. Hosmer-Lemeshow goodness of fit statistics was used to assess model fit. A 5% type-I error level was used to infer statistical significance. Receiver operating characteristic (ROC) curve analysis was done to assess baseline D-dimer level, baseline blood eosinophil count, duration of CU, CU duration at baseline, and regular antihistamine use during omalizumab treatment as potential predictors of uncontrolled urticaria.

Results

There was a total of 159 patients in the study. The median age of the patients was 43 years (IQR: 34-53); nine (6%) of the patients were over 65 years old, and 100 (63%) were female. Individuals receiving standard- or high-dose omalizumab were defined as Groups 1 and 2, respectively. Forty-one (26%) patients were obese, and the rate of obesity was similar in both groups. Asthma was the most common comorbidity, occurring in 25% of the patients, and the rate of asthma was similar in both groups. All patients received omalizumab treatment for at least 12 months. Of these, 139 (87%) were in Group 1, and 20 (13%) were in Group 2. Overall, urticaria was under control in 142 (89%) patients. The median baseline IgE level was similar in the two groups (**table I**). In Group 2, the median D-dimer level was significantly higher, regular antihistamine use during omalizumab treatment was more likely, and CU duration at baseline was shorter. Female gender, presence of angioedema, eosinopenia, basopenia, low IgE, IgG-anti-TPO positivity, duration of CU, duration of omalizumab treatment, serum CRP level, and ESR were similar in both groups (**table I**). The anti-TPO positivity was higher in patients with low IgE (31% *vs* 8%, p = 0.008). Simultaneous IgG-anti-TPO positivity and low IgE were similar in Group 1 and 2 (3% and 5%, p = 0.61). The median CU duration at baseline was shorter in Group 2 (12 *vs* 24 months, p = 0.009). Omalizumab updosing was administered at a median of 19 weeks into the treatment. The urticaria was controlled in 16 (80%) individuals in Group 2. Among controlled patients, omalizumab dose intervals were increased up to ten weeks in six (35%).

Urticaria was not controlled in a total of 17 patients, with 4 patients (20%) in Group 2 and 13 patients (10%) in Group 1. The rate of uncontrolled urticaria were similar in Groups 1 and 2 (p = 0.15). Uncontrolled patients in Group 1 were actually candidates for dose escalation. Also, 50% of uncontrolled patients in Group 2 had accompanied chronic inducible urticaria (CIndU) to CSU. In our study, cold urticaria was present in two patients, delayed pressure urticaria in two, cholinergic urticaria in four,

Figure 2 *- The association between the baseline D-dimer level and high-dose omalizumab use.*



ROC analysis revealed D-dimer level is related to high-dose omalizumab use (AUC: 0.812, p = 0.001, 95%CI [0.701-0.924]). When the cut-off is selected as 0.46 mg/dl, estimated sensitivity and specificity are 67% and 84%, respectively. AUC: area under the curve; ROC: receiver operating characteristic.

Table I *- Demographic and clinical characteristics of the study groups at baseline.*

	All patients (n = 159)	Group 1 (n = 139)	Group 2 (n = 20)	P-value
Age, median years[a]	44 (34-53)	43 (33-52)	48 (38-53)	0.33
Female, n (%)	100 (63)	88 (63)	12 (60)	0.77
BMI, mean, kg/m²	26.2 (5.1)	26.1 (5)	27.4 (5.5)	0.21
Obesity, n (%)	41 (26)	34 (24)	7 (35)	0.31
Types of chronic urticaria*, n (%)	147 (92)	135 (97)	12 (60)	NA
CSU	4 (3)	2 (1)	2 (10)	
CındU	8 (5)	2 (1)	6 (30)	
CSU+CındU				
Duration of chronic urticaria, median months	78 (42-145)	84 (44-156)	54 (34-100)	0.08
Duration of omalizumab treatment, median months	36 (18-66)	36 (17-65)	39 (23-78)	0.27
Chronic urticaria duration at baseline, median months	24 (11-81)	24 (12-88)	12 (6-24)	**0.009**
Presence of angioedema, n (%)	105 (66)	94 (68)	11(55)	0.26
MARS[2], median score	21 (19-25)	22 (20-25)	20 (18-24)	0.42
Blood eosinophil count[b], median /mm³	100 (98-258)	100 (96-265)	100 (95-189)	0.23
Eosinopenia**, n (%)	3 (2)	3 (2)	0 (0)	NA
Blood basophil count[b], median /mm³	10 (9-88)	10 (9-89)	20 (10-55)	0.41
Basopenia**, n (%)	1 (0.6)	1 (0.7)	0 (0)	NA
D-dimer level[c], median mg/L	0.19 (0.03-0.5)	0.1 (0.01-0.3)	0.75 (0.2-3)	**< 0.001**
C-reactive protein level, median mg/dl	0.6 (0.3-1)	0.5 (0.3-0.9)	0.6 (0.4-1.3)	0.45
Erythrocyte sedimentation rate, median mm/h	12.5 (4-23)	13 (4-22)	6 (3-30)	0.75
Elevated ESR, n (%)	21 (13)	17 (12)	4 (20)	0.28
ANA positivity, n (%)	4 (3)	3 (2)	1 (5)	0.45
IgG-anti-TPO positivity, n (%)	21 (13)	17 (12)	4 (20)	0.34
Baseline total IgE[d], median UI/mL	165 (65-347)	158 (61-325)	230 (70-634)	0.53
Low IgE***, n (%)	16 (10)	14 (10)	2 (10)	0.79
Both IgG-anti-TPO positivity and low IgE, n (%)	5 (3)	4 (3)	1 (5)	0.61

[a]Data is presented as mean ± SD if normally distributed, and median (interquartile range) if not normally distributed. Categorical variables are presented as number (percentages). [b]Measured in 127 patients. [c]Measured in 68 (56 [40%] in Group 1, 12 [60%] in Group 2) patients. [d]Measured in 123 patients (110 [79%] in Group 1, 13 [65%] in Group 2). *Types of urticaria are described CSU: chronic spontaneous urticaria, CındU; chronic inducible urticaria. **The cutoff value of eosinopenia and basopenia were 50×10^6 /mm³, and 10×10^6 /mm³, respectively. ***The cutoff value of low IgE level was < 43 UI/mL. [1]UCT, urticaria control test. [2]MARS, medication adherence report scale.

Table II *- Variables associated with high-dose omalizumab use, multivariable analysis.*

	RR (95%CI) *	P-value
Chronic urticaria duration at baseline, months	0.94 (0.88-1.01)	0.08
Duration of chronic urticaria, months	1.01 (0.98-1.04)	0.50
Regular antihistamine use during omalizumab treatment	3.56 (0.25-50.11)	0.35
Blood eosinophil count, median /mm³	0.99 (0.97-1.09)	0.23
D-dimer level, mg/L	4.82 (1.06-21.96)	**0.04**

*RR: Estimated relative risk and 95% confidence interval shown by odds ratio.

and symptomatic dermographism in four patients. The use of systemic corticosteroid burst in the last year was similar in the both groups after omalizumab (24% *vs* 25%, p = 0.90).

There were 16 patients with IgE levels below 43 IU/mL and only eight with IgE levels below 20 IU/mL. Of these, six patients (75%) were in Group 1, while two (25%) were in Group 2.

A positive and moderate correlation was detected between D-dimer level and high-dose omalizumab use (r = 0.43, p < 0.001). In multivariable regression analysis model, independent risk factor was D-dimer level after adjustment for blood eosinophil count, CU duration at baseline, duration of CU, and regular antihistamine use during omalizumab treatment (Nagelkerke R^2 = 0.535, **table II**). D-dimer level was significantly higher in Group 2 (**table I**). ROC analysis revealed that D-dimer level was related to high-dose omalizumab use (AUC: 0.812, p = 0.001, 95%CI 0.701-0.924). When the cutoff was selected as 0.46 mg/dl, estimated sensitivity and specificity were 67% and 84%, respectively (**figure 2**).

No adverse event related to omalizumab use was observed during 156 patient-year experience. Notably, there was a patient with uncontrolled urticaria with 600 mg of omalizumab. She was 25 years old and diagnosed with (CVID) during the follow-up, and IVIG was initiated after the diagnosis. After that, the urticaria was under control, and the omalizumab dose was decreased to 450 mg.

Discussion and conclusions

To identify factors that could predict the necessity for omalizumab dose escalation in patients with CU, a comparative analysis was conducted between 20 patients (12%) requiring high-dose omalizumab and 139 patients on standard dosing. The findings indicated that elevated D-dimer levels were independently associated with the use of high-dose omalizumab. In contrast, baseline total IgE levels, presence of low IgE, eosinopenia, basopenia, IgG-anti-TPO positivity, and obesity did not demonstrate a significant association with the need for increased dosage. Notably, the duration of CU at baseline was shorter among patients in the high-dose omalizumab group, who were also more likely to regularly use antihistamines during omalizumab treatment. Furthermore, 40% of patients in this high-dose group who remained uncontrolled also exhibited CIndU.

Asero *et al*. identified elevated D-dimer levels as a predictor of uncontrolled urticaria following omalizumab treatment, a finding that is consistent with the results of our study (22). In our cohort, an elevated D-dimer level was associated with a 4.8-fold increase in the likelihood of omalizumab updosing. Within the coagulation cascade, fibrin is degraded by plasmin, resulting in the production of fibrin degradation products and the exposure of the D-dimer antigen (23, 24). Therefore, the D-dimer level reflects both the formation (coagulation pathway) and digestion (fibrinolysis) of fibrin. This finding indicates that the coagulation cascade is activated in patients requiring high-dose treatment, and D-dimer, as the final degradation product of this pathway, can serve as an indicator for the need for high-dose omalizumab. Previous reports indicated that a higher BMI (≥ 30 kg/m²) and older age were more prevalent in the high-dose omalizumab group (21, 25). In contrast to the study by Kocatürk *et al*., our study had a larger number of patients in the standard-dose omalizumab group, while the high-dose omalizumab group comprised fewer patients (21). In the study by Curto-Barredo *et al*., the number of patients in both groups was at least twice as high as in our study. This discrepancy may account for the observed differences (25). Additionally, the study by Curto-Barredo *et al*. could not provide a clear explanation for the older age of patients in the high-dose omalizumab group.

The frequencies of comorbidities varied among the study populations (26-28). While hypertension, hypothyroidism, and allergic rhinitis have been reported as the most frequent comorbidities in various studies, asthma was the most prevalent comorbidity accompanying CU in the present study. In these three studies, the patient populations included not only those using omalizumab but also individuals treated with other medications for urticaria, such as antihistamines and cyclosporine, resulting in a heterogeneous group. Additionally, since older patients are more likely to have comorbidities, the number of elderly patients in previous studies was not clearly specified. In contrast, our study included 9 elderly patients (6%) over 65 years of age. Although hypertension and hypothyroidism are generally more common than asthma in general Turkish population, the higher prevalence of asthma compared to hypertension and thyroid diseases in our study may be attributed to the specific cohort of patients receiving omalizumab that we evaluated (29, 30). This result may also indicate that CU and asthma may have a common pathogenesis.

Omalizumab has been used off-label for CIndU. Previous studies have demonstrated its efficacy in the treatment of CIndU (6, 8). In individuals with CIndU, the rate of unresponsiveness to omalizumab was approximately one-third, and this rate was similar in both the standard-dose and high-dose omalizumab groups (31). In our study, the omalizumab dose needed to be increased in 40% of individuals with CIndU in Group 2. The response to omalizumab varies according to the subtypes of CIndU. While the response rate is higher in delayed pressure urticaria and dermographism, it can be as low as 50% in cholinergic and cold urticaria. The high rate of unresponsiveness in our study may be attributed to the higher prevalence of cholinergic and cold urticaria among the subtypes of CIndU. Additionally, CIndU is recognized as a subtype of CU that tends to be more resistant to sgAHs and omalizumab (32). Inhibitors of other molecules, such as Siglec-8, Bruton's tyrosine kinase, C5a receptor, and thymic stromal lymphopoietin, may potentially offer greater benefits than omalizumab in managing CIndU (33, 34).

Type IIb autoimmune CSU (aiCSU) is characterized by higher disease severity, concomitant autoimmune diseases, low levels

of total IgE, elevated levels of IgG-anti-TPO, basopenia, eosinopenia, poor response to antihistamines and omalizumab, and a good response to cyclosporine. In aiCSU, IgG antibodies are primarily directed against a subunit of the high-affinity IgE receptor (FcεRI), causing cross-linking of two adjacent IgE molecules and subsequent mast cell degranulation (35). Less frequently, the IgG is directed against the Fc portion of the IgE molecule itself. However, in a large, recent series of CSU patients, this autoimmune reaction was detected in less than 10% of all patients (4). A recent report by Asero *et al*. investigated the co-occurrence of IgE and IgG autoantibodies to high- and low-affinity IgE receptors (FcεRI and FcεRII), tissue factor, and thyroglobulin. The study found that more than 50% of patients had IgE and IgG antibodies to one or more of these autoantigens (36). Several, but not all, studies have reported a significantly higher prevalence of IgE-anti-TPO in patients with CSU compared to patients with autoimmune thyroid disease (0-70%) and/or healthy controls (0-8%) (37). IgE-anti-TPO and IgG-anti-TPO have been reported to be co-expressed by patients with CSU in several studies (38-42). Kolkhir *et al*. stated that the detection of IgG-anti-TPO antibodies alone is not sufficiently specific for diagnosing patients with aiCSU. However, a useful diagnostic marker for aiCSU in clinical practice is the combination of a high IgG-anti-TPO level and low IgE (37). In our study, IgG-anti-TPO positivity was comparable between both groups. Based on low levels of total IgE and IgG-anti-TPO positivity, the prevalence of aiCSU was determined to be 3%. Kohlkir *et al*. reported that 50% of individuals with aiCSU responded to omalizumab, whereas in our study, 80% of individuals with aiCSU responded to standard-dose omalizumab, and 20% required high-dose omalizumab (4).

In this study, we adopted a cutoff value of 43 IU/mL for low IgE, as defined by Ertas *et al*. among Turkish population (11). As mentioned above, low IgE can be considered a predictor of a slow or partial response to omalizumab in relation to aiCSU, and individuals with low IgE may indirectly require dose escalation of omalizumab (11, 37, 43). However, in this study, the rate of low IgE was similar in both groups. Additionally, the prevalence of individuals with simultaneous IgG-anti-TPO positivity and low IgE was also comparable between the two groups. IgE in different CSU cohorts may possess different physicochemical properties, which could explain the variations in treatment responses to omalizumab (44). Asero examined 86 patients with CSU and baseline IgE levels < 40 IU/mL, further subdividing low IgE groups (45). The highest nonresponse rate, at 85%, was observed in the group with IgE levels below 20 IU/mL. However, in our study, there were 16 patients with IgE levels below 43 IU/mL and only eight with IgE levels below 20 IU/mL. Of these, six patients (75%) were in the standard-dose omalizumab group, while two were in the high-dose group. Therefore, 25% of the eight patients with IgE levels below 20 IU/mL were nonresponsive to the standard dose. When adopting a 20 IU/mL cutoff as per the study by Asero, we found that not only was the number of patients too low for a meaningful comparison, but also that 25% of these patients were nonresponsive. These findings may not support the data reported by Asero possibly due to the smaller sample size when using the same cutoff value for low IgE.

In a study conducted by Kolkhir *et al*. eosinopenia was associated with high disease activity in patients with CSU. High CSU activity was observed in one-third of patients with eosinopenia and/or basopenia, compared to 15% of patients with normal or elevated eosinophil levels (14). Unlike this study, eosinopenia and basopenia were not observed in Group 2. Eosinopenia and basopenia predicted non-response to omalizumab in CSU patients with high specificity and moderate to low sensitivity (14). However, further studies are needed to achieve higher sensitivity. In our study, eosinopenia and/or basopenia were not predictive factors for the response to standard-dose omalizumab.

Although some publications suggest that elevated ESR may be associated with high disease activity, aiCSU, and unresponsiveness to standard-dose omalizumab, data on the relationship between omalizumab dose escalation and elevated ESR are limited (46, 47). Our results indicated that baseline ESR and elevated ESR were similar in both groups, and neither appeared to predict the need for omalizumab dose escalation.

CU duration at baseline was not a predictor for omalizumab dose escalation, although it was shorter at baseline in Group 2, which is consistent with the current literature (9, 21).

The EAACI/GA²LEN/EuroGuiDerm/APAAACI guidelines recommend evaluating the effectiveness of standard-dose omalizumab at 16 or 24 weeks (20). In our study, dose escalation was administered at a median of 19 weeks, indicating a relatively long dose escalation period. As this was a real-life study, the timing of dose escalation may have changed due to differences in patient perceptions of control, trigger factors, accompanying comorbidities, adherence to control visits, challenges within the healthcare system (25). Our cohort includes a heterogeneous group of patients in Group 1 who had minimal, partial, or no response to standard-dose omalizumab. Consequently, a significant number of patients might have continued on the standard dose for an extended period with a partial response. Additionally, a personalized treatment approach to dose escalation may have further prolonged the duration.

The present study has several limitations. Compared to the standard-dose omalizumab group, the number of patients in the high-dose omalizumab group was relatively smaller. Since this was an observational real-life study, the omalizumab dose may have been increased for patients with partial or poor responses in the standard-dose group. Consequently, not all patients in the high-dose group were entirely unresponsive.

In conclusion, D-dimer level seems to be a predictor for omalizumab updosing. IgG-anti-TPO positivity and low IgE do not predict the need for dose escalation; however, these results should be strengthened with a larger number of patients.

Previous presentations

GT presented as a poster presentation at the XXIX National Allergy and Clinical Immunology Congress between 29 November-3 December 2023, and presented as a poster in UCARE Conference between 7-9 December 2023, São Paulo, Brazil.

Fundings

None.

Contributions

GT: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, supervision, visualization, writing – original draft, writing – review & editing. ED: formal analysis, project administration, supervision, visualization, writing – original draft, writing – review & editing; GK, AFK: supervision, visualization, writing – original draft, writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

GT would like to express her gratitude to Esra Demirtas and Ferdane Karaman for their technical support in managing patient records during visits and injection applications in our clinic.

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ORIGINAL ARTICLE

Eur Ann Allergy Clin Immunol Vol 58, N.2, 77-85, 2026

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Severe chronic spontaneous urticaria responding and not responding to omalizumab: analysis of the prognostic value of known and novel *in vitro* variables

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Key words

Chronic urticaria; omalizumab; biomarkers.

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Doi
10.23822/EurAnnACI.1764-1489.375

Impact statement

In severe CSU baseline D-dimer, IgE and IgG anti-FceRI, and ECP are elevated while soluble MRGPRX2 is low. One week after omalizumab start IgE anti-FceRI and D-dimer drop in early responders, while SP increases in all patients.

Summary

Background*. Chronic spontaneous urticaria (CSU) response to anti-IgE treatment can be rapid, late or absent. Recently, potential mechanisms of activation of mast cells alternative to FceRI, including mas-related G protein-coupled receptor X2 (MRGPRX2), activation of coagulation cascade, and activation of eosinophils have been described. We measured several potential in vitro markers, including well-known MRGPRX2 activators, in sera of patients CSU both responding and not responding to omalizumab.* ***Methods.*** *D-dimer, substance P (SP), eosinophil cationic protein (ECP), soluble MRGPRX2, IgE anti-FceRI, IgE anti-FceRII, IgG anti-FceRI and IgG anti-FceRII were measured in 32 patients with severe CSU at baseline and one week after the start of omalizumab therapy, and in 20 healthy controls.* ***Results****. At baseline CSU patients showed significantly higher levels of D-dimer, IgE anti-FceRI, IgG anti-FceRI, and ECP ($p < 0.001$ in all cases), and significantly lower levels of soluble MRGPRX2 ($p = 0.009$) than controls. The two groups showed similar levels of IgG and IgE to FceRII and SP. One week after the first omalizumab administration there was a significant drop of IgE anti-FceRI ($p < 0.001$) and D-dimer ($p = 0.028$), in early responders. SP increased in all CSU patients ($p < 0.001$) irrespective of the final response to omalizumab. IgE anti-FceRI response at one week was associated with the final response to omalizumab (OR 0.12 [95%CI 0.01-1.06]).* ***Conclusions****. Severe CSU is associated with high plasma levels of several biomarkers including D-dimer, IgE anti-FceRI, IgG anti-FceRI and ECP and low levels of soluble MRGPRX2. IgE anti-FceRI response at one week may predict the final response to omalizumab.*

Introduction

The current international guidelines recommend second generation antihistamines at licensed dosage, at up to 4-fold the licensed dosage, and omalizumab as steps I-III in the treatment of chronic spontaneous urticaria (CSU) (1). Although a majority of patients gets complete or at least partial control of the disease by this stepwise treatment, about 10% of severe CSU patients seem totally unresponsive and have to be shifted to the treatment with cyc-

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losporin. In a series of 296 antihistamine-resistant CSU patients treated with omalizumab at the Clinica San Carlo, 27 (9%) had to be shifted to cyclosporin treatment, always with excellent clinical responses (unpublished). Patients responding slowly or not responding to omalizumab are generally characterized by low total IgE levels (2), a feature that has been associated with autoimmune chronic spontaneous urticaria in the international PURIST study (3). Notably, in omalizumab-refractory patients IgE levels are lower than in partial/late responders to the drug (2). Anti-IgE therapy leads to a progressive downregulation of the high affinity IgE receptor that parallels the reduction in number of both free and membrane-bound IgE. Thus, if mast cell activation occurred always via the high affinity IgE receptor, one is tempted to speculate that in the minority of omalizumab-refractory patients the activation of effector cells might occur mainly by pathways that bypass the FceRI which needs the presence of autoimmune IgE (4) or autoimmune IgG directed against membrane-bound IgE or FceRI (5) to be activated. Recent studies have highlighted the potential relevance of MRGPRX2 receptor as an activation pathway in patients with CSU. MRGPRX2 is a G-coupled human mast cell receptor constitutively expressed by human skin mast cells which mediates non-immune adverse reactions without the involvement of antibody priming (6). MRGPRX2 is activated by several different substances, including neuropeptides such as Substance P (SP), as well as by mediators of eosinophils, namely eosinophil cationic protein, major basic protein and eosinophil peroxidase (7, 8). Other mechanisms of mast cell activation potentially viable in CSU beyond the high affinity IgE receptor, including the coagulation cascade, the complement system, and the platelet activating factor (PAF) vicious circle, have been recently reviewed in detail (9, 10). Interestingly, during the last years, it was demonstrated that in CSU both eosinophils and endothelial cells are often activated and are potentially able to induce the histamine release from mast cells through different mechanisms, including the activation of the extrinsic pathway of the coagulation cascade by an hyperexpression of tissue factor (11-13). Starting from these observations, we measured several potential *in vitro* markers, including well-known MRGPRX2 activators, in sera of patients CSU both responding and not responding to omalizumab.

Patients and methods

Patients

Thirty-two patients with severe CSU, *i.e.*, urticaria activity score on 7 days (UAS7) ≥ 20, refractory to second generation antihistamines even at higher than licensed dosage, underwent subcutaneous omalizumab treatment at a dose of 300 mg every 4 weeks, following the indications of the European Academy of Allergy and Clinical Immunology (EAACI) guidelines (1). Following the prescriptions by the Italian national regulatory agency (AIFA) that prohibit up-dosing and prevent the continuation of the treatment in the absence of a significant clinical response after 3 administrations, the patients were evaluated at baseline and then every 4 weeks for 12 weeks after the omalizumab initiation, and UAS7 was recorded at each time point. An early response was defined as the achievement of an UAS7 score ≤ 6 within 4 weeks after the first administration, a late response was defined as the achievement of the same reduction within 12 weeks after initiation, while a non-response was defined as the persistence of unchanged symptoms 4 weeks after the third administration (UAS7 ≥ 10). The patients were investigated at baseline and, due to funding shortage, only 1 week after omalizumab administration for the circulating levels of D-dimer, substance P (SP), eosinophil cationic protein (ECP), Mas-related G protein-coupled receptor X2 (MRGPRX2), IgE anti-FceRI, IgE anti-FceRII, IgG anti-FceRI and IgG anti-FceRII. Twenty healthy subjects, sex- and age-matched with patients, served as controls.

The study was conducted according to the ethical principles of the Declaration of Helsinki and the code of Good Clinical Practice. The patients and controls gave informed written consent to the use of their sera and relative data. The local review board approved the study.

Methods

D-dimer levels were measured in Na-citrated plasma with a commercial ELISA method (Zymutest D-dimer, Hyphen Biomed, Neuville sur Oise, France). A highly purified monoclonal antibody specific for D-dimer is absorbed on wells of microplates. The analyte captured onto the solid phase is detected by a monoclonal antibody coupled to horse radish peroxidase. The intra- and inter-assay coefficients of variation (CV) were 10 and 15%, respectively. The lower detection limit was 0.22 μg/mL.

Eosinophil cationic protein (ECP) plasma levels were measured by a commercial sandwich enzyme immunoassay (RNASE3) from Cloud-Clone Corp, Katy. TX, USA. The analyte is captured by a specific antibody adsorbed to the microtitration plate and detected by a second specific biotin-conjugated antibody. Intra- and inter-assay CV are 10% and 12%, respectively. The lower detection limit was 29 pg/mL.

Substance P (SP) plasma levels were measured by a commercial competitive inhibition enzyme immunoassay from Cloud-Clone Corp, Katy, TX, USA. A monoclonal antibody specific to SP has been pre-coated onto a microtitration plate. A competitive inhibition reaction is launched between biotin labeled SP and unlabeled SP (standards or samples) with the pre-coated antibody specific to SP. After washing, the biotin labeled SP is detected by avidin conjugated to horseradish peroxidase. Intra- and inter-assay CV are 10% and 12%, respectively. The lower detection limit was 4.99 pg/mL.

Mas-related G-protein coupled receptor member X2 (MRGPRX2) plasma levels were measured by a commercial sandwich

ELISA kit from Assay Genie, Dublin, Ireland. Capture antibody is pre-coated onto the microtitration plate. The analyte is detected by a biotin conjugated specific antibody. Intra- and inter-assay CV are 8% and 10%, respectively. The lower detection limit was 0.938 ng/ml.

IgE and IgG anti- FCeRI and anti- FCeRII plasma levels were measured by home-made sandwich ELISAs. Recombinant human FCeRI (Sino Biological, Eschborn, Germany) or recombinant human FCeRII (Abcam, Cambridge, UK) were coated overnight onto microtitration plates at a concentration of 10 µg/mL. After washing, the residual binding sites were blocked with bovine serum albumin 1%. After further washes, serum dilutions (1:10) from patients and controls were added and incubated for 45 minutes at 37 °C. For the determination of specific IgE, after washing, we added goat anti-human IgE (ε chain specific) (Sigma, St. Louis, Mo, USA). After a further incubation of 45 minutes and washing, donkey polyclonal anti-goat IgG peroxidase conjugated (Santa Cruz Biotechnology Inc, Dallas, Texas, USA) was added and incubated for 45 minutes. For the determination of specific IgG, we added a goat anti-human IgG (ε-chain specific) HRP conjugate (Sigma, St. Louis, Mo, USA) and incubated for 45 min. All the reactions were revealed by orthophenylenediamine (Sigma Chemical, St. Louis, Mo, USA). Both intra- and inter-assay CVs are less than 20%.

With the exception of D-dimer, all measurements were carried out in EDTA-anticoagulated plasma.

Statistics

The results are expressed as the medians and ranges (minimum-maximum). A Mann-Whitney U test was used to compare different groups and controls, whereas Wilcoxon-signed rank test was used to compare patients at different times. The correlations were assessed by means of Spearman's rho. P-values below 0.05, two-sided, were considered statistically significant. The results are expressed as the medians and ranges (minimum-maximum). Univariate logistic models were fitted to identify prognostic factors on response including baseline and post therapy parameters: D-dimer, ECP, substance P, MRGPRX2 levels, IgE anti-FceRI, IgE anti-FceRII, IgG anti-FceRII and IgG anti-FceRII. Forest plot of univariate analysis was built. ROC curves were calculated to evaluate model performance. (IBM SPSS Statistics for Windows, version 29.0, IBM Corp., Armonk, NY, USA and Stata 18, StataCorp, College Station, TX, USA).

Results

Patients at baseline

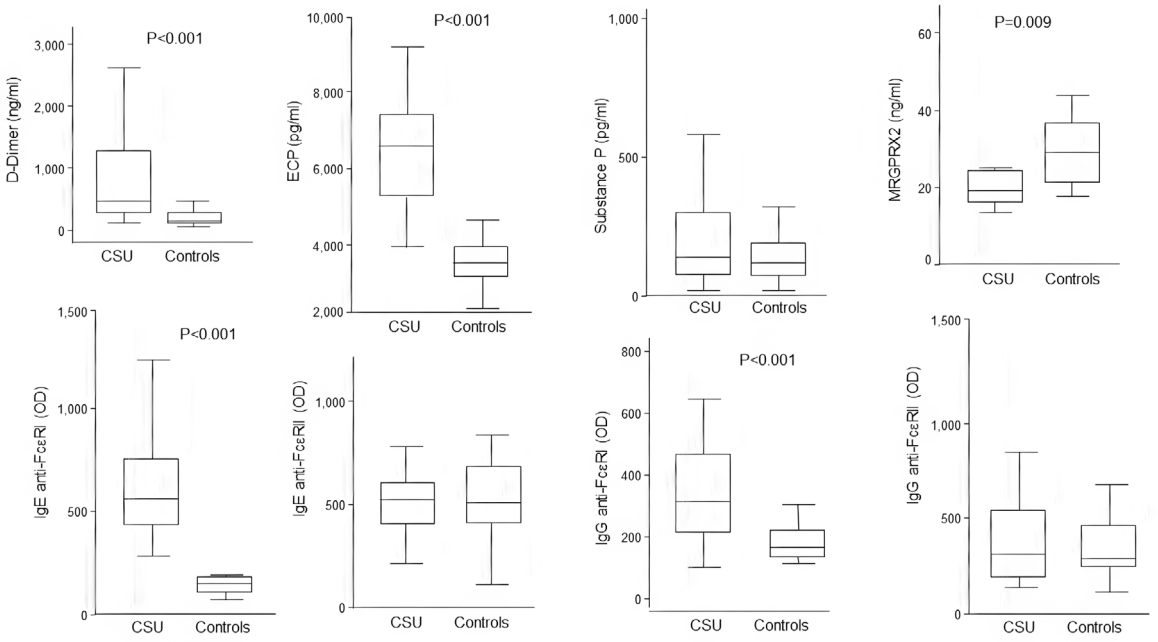
The demographics and clinical features of patients are summarized in **table I**. All 32 CSU patients showed a UAS-7 of 20 or more at baseline, and 17 of them had also angioedema. The most common comorbidities were atopy (9/32) and thyroiditis (5/32). Patients showed a median age of 56 years (range 21-87), a M/F distribution of 11/21, and a median disease duration of 4 years (2-7). All patients showed an UAS-7 of 20 or more at baseline. Healthy controls showed a median age of 54 years (20-81) and a M/F ratio of 7/13 (p = NS).

Figure 1 shows the comparison between CSU patients at baseline and healthy controls for parameters potentially involved in CSU pathophysiology. At baseline, CSU patients showed significantly higher levels of D-dimer (median 473 ng/ml [range 121-4530]) than healthy controls (147 ng/ml, [55-476] ng/ml) ($p < 0.001$). We also found higher levels of IgE anti-FceRI (562 OD, [283-1421] *vs* 151 OD, [73-455]) ($p < 0.001$), IgG anti-FceRI (315 OD [102-888] *vs* 160 OD [96-294]) ($p < 0.001$) and ECP (6595 pg/ml, [3,960-9,181] *vs* 3,115 pg/ml, [1,932-4,252]) ($p < 0.001$). In contrast, MRGPRX2 levels were lower in CSU patients at baseline (16 ng/ml [10-76]) than in normal controls (26 ng/ml, [15-41]) (p = 0.009). The two groups did not differ in the levels of IgG and IgE to the low affinity IgE receptor, FceRII, as well as in SP levels.

Table I *- Demographic and clinical features of chronic spontaneous urticaria (CSU) patients.*

	CSU patients (n = 32)	**Healthy controls (n = 20)**
Age - Median (range)	56 (21-87)	54 (20-81)
Male/Female	11/21	7/13
UAS-7 - Median (range)	31 (20-42)	
Duration of disease – median years (range)	4 (2-7)	
Angioedema (n / total)	17 / 32	
Atopy (n / total)	9 / 32	
Thyroiditis (n / total)	5 / 32	
Eosinophils n/mL– Median (range)	100 (40-800)	100 (30 -350)

Figure 1 - *Plasma levels of D-dimer, eosinophil cationic protein (ECP), substance P, soluble mas-related G-protein coupled receptor member X2 (MRGPRX2), IgE and IgG anti-FCeRI and anti-FCeRII in 32 patients with severe chronic spontaneous urticaria (CSU) at baseline and 20 healthy controls. Results are expressed as median values, interquartile ranges (boxes), and 5th and 95th percentiles (whiskers).*



Patients at baseline divided on the basis of the final response to omalizumab

Based on omalizumab response, 13 patients were considered early responders, 9 late responders and 10 non responders. The baseline values of the various parameters in the three subgroups are reported in **table II**. No significant difference was evident among the three subgroups for all the parameters tested at baseline (**tables I, II**). Concerning comorbidities, among the 13 early responders, 3 were atopic and 1 had thyroiditis; among the 9 late responders, 3 were atopic and 1 had thyroiditis; and among the 10 non-re-

Table II - *Values of parameters involved in the pathogenesis of CSU at baseline and after one-week therapy with omalizumab in 32 CSU patients divided according to the final response to omalizumab.*

	Baseline			One-week therapy		
	Early responders	**Late responders**	**Non responders**	**Early responders**	**Late responders**	**Non responders**
D-Dimer - ng/ml	393 (121-2,623)	370 (157-4,530)	378 (191-1,781)	244 (83-722)*$	350 (179-3,784)	378 (191-1,781)
ECP - pg/ml	6,392 (3,987-8,394)	7,323 (4,766-8,797)	6,595 (3,960-9,181)	5,918 (3,262-7,832)	6,900 (3,461-8,202)	5,613 (3,839-7,860)
Substance P - pg/ml	171 (52-581)	235 (46-1,114)	119 (20-321)	319 (51-909)$$	400 (70-1,586)$$	591 (134-1,060)$$
MRGPRX2 - ng/ml	15.8 (10.4-53.7)	16.0 (11.7-76.4)	18.0 (11.2-40.3)	15.1 (6.5-23.6)	17.9 (11.1-69.8)	18.5 (10.0-36.9)
IgE anti-FceRI - OD	612 (370-1,421)	557 (283-1,043)	475 (400-1,399)	314 (255-622)**$$	398 (230-566)	391 (261-1,137)
IgE anti-FceRII - OD	497 (353-688)	560 (201-667)	521 (207-1,306)	521 (344-651)	575 (192-700)	572 (218-1287)
IgG anti-FceRI - OD	282 (102-583)	304 (121-888)	417 (161-882)	268 (78-565)	263 (100-738)	484 (93-890)
IgG anti-FceRII - OD	282 (154-668)	356 (175-754)	377 (144-1,618)	279 (137-676)	372 (168-709)	234 (153-1391)

* = 0.028 vs baseline; $ = 0.05 vs non responders; $$ = < 0.001 vs baseline; ** = 0.045 vs non responders.

sponders, 3 were atopic and 3 had thyroiditis. The duration of CSU was unrelated to omalizumab response.

Patients one-week after the first omalizumab administration divided on the basis of final response

The values of the various parameters one week after the first injection of omalizumab are reported in **table II**. In early responders, already one week after the first omalizumab administration, the levels of D-dimer dropped significantly (244 ng/ml [83-722]) from baseline (393 ng/ml [121-2623]) ($p = 0.028$) and were significantly lower than in non-responders (378 ng/ml, [191-1781]) ($p = 0.05$). D-dimer level was not able to discriminate between late and non-responders one week after the start of treatment. A similar behavior in early responders was observed for the levels of

Figure 2 - (A) *Upper part: plasma levels of IgE anti-FCεRI and D-dimer in patients with severe chronic spontaneous urticaria at baseline and one-week after the first omalizumab administration divided on the basis of final response. Lower part: clinical response to omalizumab, expressed as urticaria activity score on 7 days (UAS7);* ***(B)*** *Plasma levels of IgE anti-FcεRI at baseline and after one week from the first administration of omalizumab in patients with severe chronic spontaneous urticaria divided on the basis of final response to the drug.*

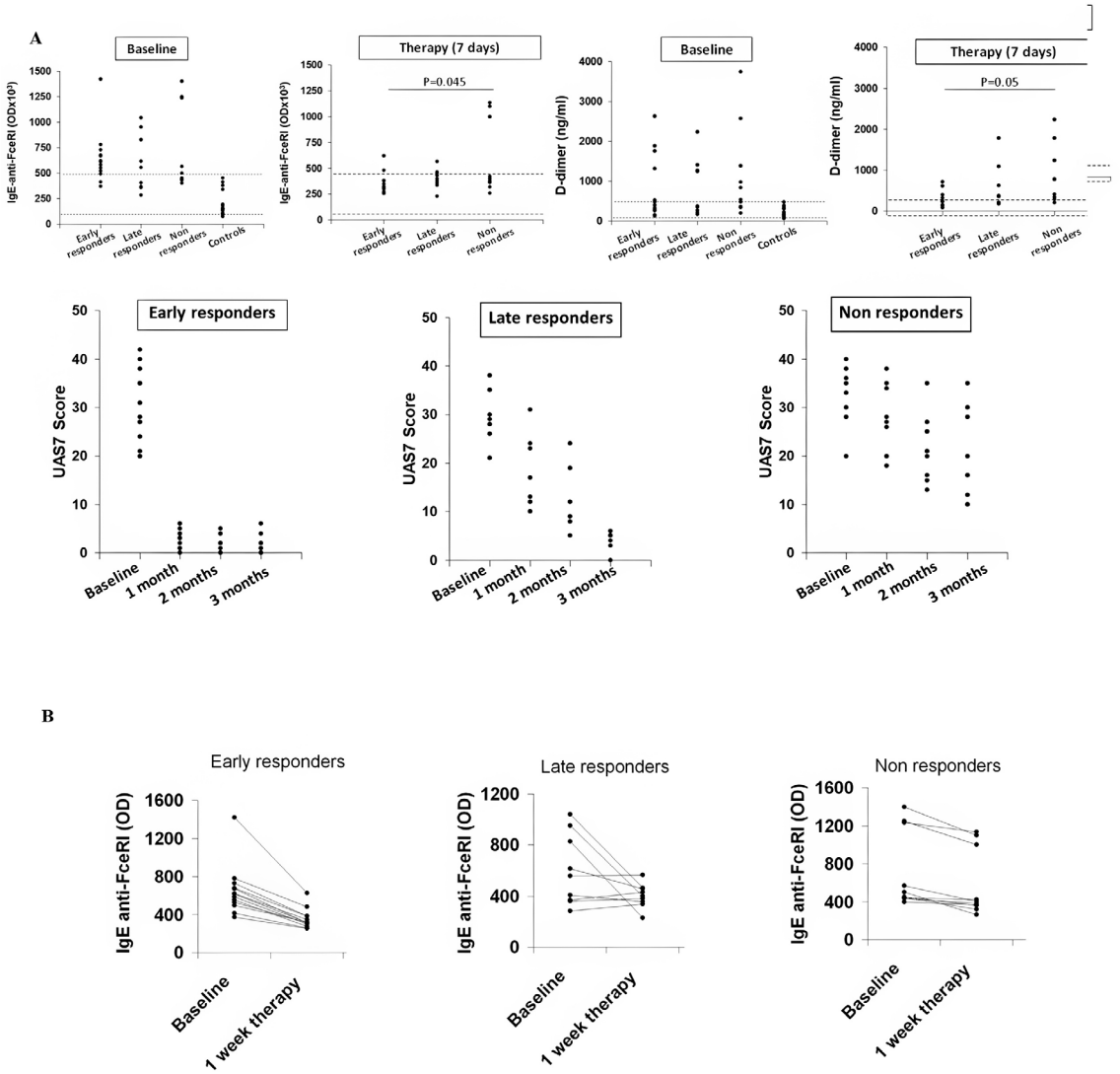
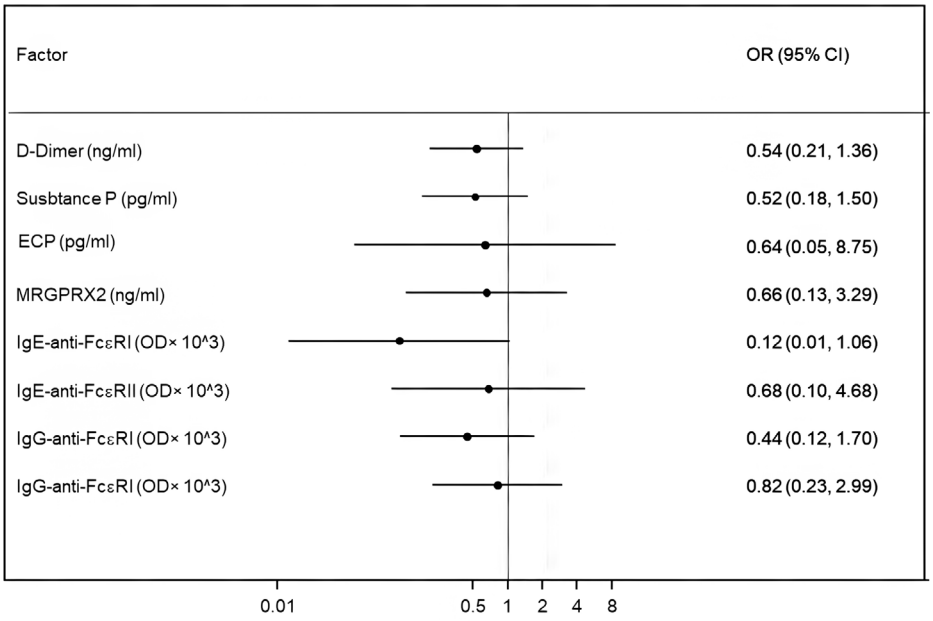


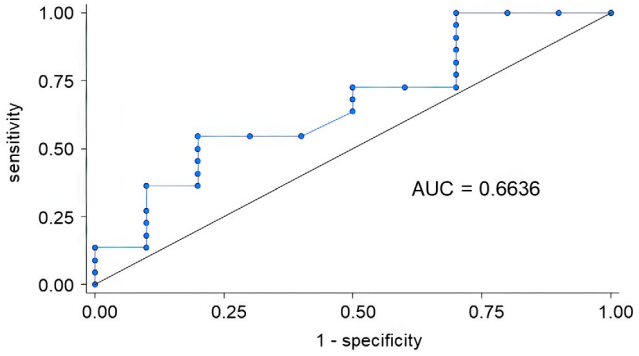
Figure 3 *- Evaluation of the association between the response of the various parameters after one week from the first administration of omalizumab and the final clinical response to the drug.*



Logistic regression analysis shows that IgE anti-FceRI levels after one week of therapy were significantly associated with a final good response to omalizumab, with an odds ratio (OR) of 0.12 (95%CI 0.01-1.06).

IgE to FceRI, which significantly decreased from 612 OD (370-1421) to 314 OD (255-622) after one week and were significantly lower than in non-responders (391 OD, [261-1137]) (p = 0.045) (**table I** and **figure 2A**). Again, no statistically significant difference was observed between late- and non-responders one week after the start of the treatment. Levels of substance P significantly increased in all CSU patients one week after omalizumab administration from 140 [20-1,114] to 395 [51-1586] (p < 0.001), without any difference among the three subgroups. Finally, the three subgroups did not show significant differences in the levels of ECP, MRGPRX2, IgE-anti-FceRII, IgG-anti-FceRI and IgG-anti-FceRII one week after the start of anti-IgE treatment.

Figure 4 *- Receiver operating characteristic (ROC) curve relative to the IgE anti-FcεRI response after one week of omalizumab therapy, showing a good capacity to discriminate between responders and non-responders as indicated by an area under the curve (AUC) of 0.66 (95%CI 0.45-0.88).*



Prediction of the final response to omalizumab by different factors at baseline and after one-week therapy

Logistic regression analysis showed no association of the parameters at baseline with the final response to omalizumab. In contrast, one week after the start of biological therapy, the reduction of IgE anti-FceRI (**figure 2B**) was significantly associated with a final good response to omalizumab (**figure 3**), with an odds ratio (OR) of 0.12 (95%CI 0.01-1.06). Considering again the IgE anti-FceRI response after one week of omalizumab therapy, the ROC (receiver operating characteristic) curve showed a good capacity to discriminate between responders and non-responders after three months of therapy as indicated by an area under the curve (AUC) of 0.66 (95%CI 0.45-0.88) (**figure 4**).

Discussion and conclusions

In the present work we studied several potential mast cell activators in patients with severe CSU with the aim to detect whether differences existed between patients and normal control, between different patients' subgroups, and whether omalizumab-refractory patients could be identified before the start of the treatment.
Concerning D-dimer plasma levels, we confirmed our previous findings that D-dimer represents an excellent marker of severity of CSU, as its levels were significantly more elevated in our patients than in normal controls irrespective of the final clinical response to omalizumab (14). At the same time, we confirmed also that D-dimer level is an excellent marker of disease activity as it strictly parallels the clinical response to CSU treatment (15, 16). What was still unknown is the rapid drop of elevated D-dimer in response to anti-IgE treatment, that was already significant as short as one week after the first omalizumab administration. This demonstrates that in CSU the activation of the coagulation cascade is an exquisitely inflammatory process that stops suddenly as soon as the mast cell activation ceases.
In this study, we did not evaluate other potential predictors of response to omalizumab, such as anti-TPO autoantibodies and total IgE, which we had assessed in a previous study (17). That study concluded that thyroid autoimmunity alone cannot serve as a clinical predictor of response to omalizumab, whereas total IgE levels remain the most reliable prognostic marker for omalizumab response in patients with severe CSU (17). Other authors found that the IgG anti-TPO/total IgE ratio may be a good predictor of omalizumab response (18, 19). Similarly, the autologous serum skin test (ASST) was not carried out in the patients included in the present work; however, a recent prospective study performed by one of us demonstrated that a positive ASST predicts a slow response to Omalizumab (20).
The analysis of the levels of FceRI IgE in plasma confirms that type I autoimmunity is largely prevalent in CSU patients, irrespective on their response to omalizumab (21, 22), although IgE levels gradually decreased from early omalizumab responders to late and non-responders. Omalizumab response is probably largely influenced by the co-occurrence of autoimmune IgG to the high affinity IgE receptor (21, 22). In effect, recent studies showed that autoimmune CSU is in most cases associated with auto-allergic immune reactivity, but not vice-versa (23). Interestingly, but not surprisingly, the levels dropped as soon as one week after omalizumab administration, which indirectly confirms the good quality of our data and the rapidity of action of the drug.
The levels of autoimmune IgG to the high affinity IgE receptor (IgG-anti-FceRI), that are considered responsible for histamine release in patients with autoimmune CSU (3), increased gradually from early omalizumab responders up to late and non-responders. These auto-antibodies were missing in normal controls. Not surprisingly the levels of circulating IgG autoantibodies to the high affinity IgE receptor did not change one week after the first administration of the anti-IgE mAb.
IgG-anti-FceRII autoantibodies have been considered as part of the type IIb autoimmune process in CSU as activators of eosinophils (24). Interestingly they were detected mainly in late and non-responders to omalizumab although at low levels (**table II**).
ECP, a marker of eosinophil activation, was elevated in CSU patients irrespective of the final omalizumab response. This finding confirms our previous observations about eosinophil activation as a turning point in the expression of both tissue factors, that causes the activation of the coagulation cascade by the extrinsic pathway, and of vasoactive substances such as VEGF (12, 25, 26). Another important point to keep in mind is that ECP is able to activate mast cells via the MRGPRX2 receptor.
Substance P, which was investigated as one of the several substances able to activate mast cells by membrane MRGPRX2 receptor, was not elevated in our patients irrespective on their response to omalizumab. This finding fully confirms the result of an old study by our group (27), although other groups got different results (28). To our surprise, the plasma levels of substance P increased dramatically in the same patients as short as one week after the first administration of omalizumab, irrespectively of the final clinical response to the drug. A similar trend of substance P was previously observed in CSU at 3 and 6 months of therapy in two Turkish studies (29, 30). One could speculate that, since substance P is an activator of mast cells via the MRGPRX2 receptor, the down-regulation of mast cell function may eventually lead to a release of substance P from the receptors they are bound to.
Soluble MRGPRX2 showed uniformly low levels in all CSU subsets, and such levels were even lower than those detected in normal controls. This finding is quite surprising, in view of previous studies reporting a hyper-expression of Mas-related gene X in skin mast cells (31), and the detection of elevated MRGPRX2 serum levels in patients with severe CSU positively correlated with UAS7 (32). Our finding might theoretically suggest that the plasma levels of the soluble form of this receptor are inversely related to the degree of activation of the mast cell or, in other words, that strongly

activated mast cells retain the MRGPRX2 on their surface as a highly expressed membrane receptor. Such levels did not increase one week after omalizumab administration, irrespective of the final response to the drug. It would be interesting to re-measure MRGPRX2 levels in CSU patients stably in clinical remission.

In summary, our study confirms the relevance of D-dimer as a non-specific marker of severity and of acute inflammation in a proportion of severely affected patients. It also shows the rapid drop in circulating IgE after omalizumab administration, which may explain the nearly immediate clinical response observed in some CSU patients. On the other hand, the low levels of circulating MRGPRX2 and of SP at baseline were unexpected; particularly impressive was the increase in SP levels as short as 7 days after the first omalizumab administration. Following-up our *in vitro* variables for longer that one week would have been ideal, but due to funding shortage this was not possible. IgE anti-FcεRI response at one week might be proposed as one further marker to predict the response to omalizumab. However, this assay is not present in most settings, while other *in vivo* and *in vitro* assays such as the autologous serum skin test, total IgE levels, or thyroid autoimmunity are much more commonly available. Although we were not able to detect novel markers of response to omalizumab and larger studies are needed to confirm our findings, we believe that this study, albeit with its evident limitations, may open new pathways in the understanding of the complex immune-pathogenesis of CSU.

Fundings

None.

Contributions

RA: conceptualization, project administration, supervision, writing - original draft, writing - review & editing. PC, SF: data curation, investigation. ML, VC: methodology, validation. MC: conceptualization, project administration, supervision, review & editing. SS, DC: formal analysis.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

Eur Ann Allergy Clin Immunol Vol 58, N.2, 86-96, 2026

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Early markers of baked milk and egg tolerance in young children with IgE-mediated immediate reactions

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Key words

Baked milk; baked egg; early tolerance; IgE-mediated immediate-type allergy; infant.

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Doi
10.23822/EurAnnACI.1764-1489.383

Impact statement

The study focuses on a unique particular subset. It identifies prognostic markers during the initial reactions and delineates the group for whom a very early reduction of dietary restrictions may be feasible.

Summary

Background. *Children with milk and egg allergies have outcomes in which three-quarters are tolerant to baked forms of the allergenic food. Identifying predictors of tolerance to baked foods for IgE-mediated immediate-type reactions may guide the early introduction of baked allergens to diet and tolerance development. This study explores factors associated with early tolerance to baked foods.* ***Methods.*** *We retrospectively analyzed patients with IgE-mediated immediate-type food allergy in infancy who either became tolerant to the baked form before two years or remained reactive after two years.* ***Results.*** *We examined 143 patients solely with IgE-mediated immediate-type egg and/or milk allergies excluding those with atopic dermatitis. 76 (42 egg-allergics; 34 milk-allergics) achieved tolerance, and 67 (38 egg-allergics; 29 milk-allergics) were reactive beyond the age of two. Receiver operating characteristic analysis determined cut-off values for specific-Immunoglobulin E (sIgE) levels (kU/L) predicting mild phenotype at first admission: egg white-sIgE ≤ 7.39, milk-sIgE ≤ 5.99, and casein-sIgE ≤ 4.99, with AUC values of 0.703, 0.716, and 0.749, respectively.* ***Conclusions.*** *This study identifies key prognostic factors for tolerance to baked allergen for IgE-mediated immediate-type reactions, providing valuable insights to determine the patients who need more intensive care versus the ones who don't need baked allergen avoidance early in their life from their initial admission at infancy.*

Introduction

Milk and egg are the leading causes of food allergy in infants and young children. The estimated prevalence rates range from 0.9% to 3.8% for milk and 1% to 2% for egg, respectively, although these numbers vary across different populations (1-4). Food allergies pose a significant public health concern due to their frequency in the population and potential to cause life-threatening reactions. Although many patients outgrow their food allergies within the first few years of life, a significant subset continues to experience allergic reactions into adolescence and even adulthood. This refractory course necessitates ongoing management and vigilance to prevent and treat potential allergic reactions (5-10). Predicting whether and when an allergy will resolve spontaneously is often challenging. Various factors have been linked to the persistence of food allergies, including a history of anaphylaxis, the severity of reactions, the age at symptom onset, family history of atopy, specific immunoglobulin E (sIgE) levels, skin prick test wheal sizes, the presence of multiple food allergies, and the involvement of multiple systems. These factors contribute to

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the complexity of managing food allergies and highlight the need for tailored approaches to patient care (6, 7, 9).

In recent years, IgE-mediated milk and egg allergies have been graded based on the tolerability of baked products containing these allergens. Approximately three-quarters of the affected population can tolerate baked products and are more likely to outgrow their allergies. Conversely, those who react to baked milk (BM) and baked egg (BE) are more likely to have persistent allergies (11-16). The structure of milk and egg proteins can be altered by heat and the food matrix. Incorporating the offending food into the dough and baking it changes the antigenic structures. Children with IgE antibodies directed against 3D conformational epitopes are likely to tolerate the antigenic food after it undergoes this cooking process (17-19). This tolerability offers significant social, practical, and nutritional benefits to children by allowing a less restricted diet. In addition to these advantages, continuous consumption of the tolerated form of the allergenic food (baked milk or baked egg) may promote immunological tolerance to the unbaked forms, thus enhancing the overall patient experience. This approach can lead to an improved quality of life and potentially expedite the resolution of the allergy (20).

Upon diagnosing a food allergy, the standard practice is to eliminate all forms of the allergenic food, including its baked variants, from the diet until a physician-supervised challenge can be conducted (21). Predicting whether a patient will tolerate baked foods in the future remains challenging, but various diagnostic approaches have been proposed. The potential utility of skin prick testing and specific IgE measurements has been explored, typically focusing on results obtained closer to the time of an oral food challenge (OFC) (13, 22-32). These methods aim to improve the accuracy of predicting baked food tolerability and guiding patient management strategies (13, 22-31).

The first two years of life represent a crucial window during which infants can develop tolerance to previously allergenic foods. However, data on the rates of tolerance to baked forms within this period is limited. Research has primarily focused on whether tolerating baked foods impacts the resolution of allergies to their native forms (9, 11). In this study, we investigate the natural history of infants admitted solely with immediate-type hypersensitivity to milk and/or egg with a specific focus on their tolerability to baked forms of these foods by the age of two in addition to their predicting factors for baked allergen tolerability at initial admission.

Materials and methods

The study retrospectively analyzed medical records of patients diagnosed with food allergy at the Pediatric Allergy and Immunology Unit of the University Hospital between January 2020 and January 2023. The study was conducted per good clinical practice rules and received ethical approval from the Marmara University Ethical Committee (Protocol ID: 09.2024.222). The ethics committee did not deem it necessary to obtain informed consent from participants for this study.

We reviewed data from 564 patients diagnosed with milk and/or egg allergies. Eligibility criteria included the onset of an allergic reaction to the offending food within the first 12 months of life. Inclusion criteria encompassed a clear history of an immediate reaction within two hours of ingestion of milk or egg, presenting with symptoms such as urticaria and/or angioedema, wheezing, coughing, dyspnea, stridor, vomiting, anaphylaxis (33, 34), or a positive oral food challenge (OFC) under physician supervision. A positive sIgE test was defined as milk-sIgE or egg white-sIgE ≥ 0.35 kU/L to the trigger food within three months of the initial reaction.

Following these criteria, out of 564 patients whose records were screened, exclusions were made for the following reasons: absence of an immediate type reaction (n = 308), history of a first IgE-mediated rapid reaction occurring after the age of one year (n = 31), lack of evidence for a positive sIgE to the trigger food in the medical records within three months of the initial reaction (n = 25), missing regular outpatient clinic records every 3-6 months until at least two years of age (n = 37), and presence of moderate or severe atopic dermatitis (AD) at the time of diagnosis (n = 20) (35). These patients were excluded to ensure the integrity and specificity of the study cohort.

Data collection and oral food challenge test (OFC)

Patients' demographic, clinical, and laboratory data were collected from medical records. This data included gender, age, age at onset, type of initial reaction, presence of other food allergies identified before or at the time of the first reaction, and family history of atopy (diagnosed by a physician). Initial laboratory values were also gathered, including milk-sIgE, casein-sIgE, egg white-sIgE, total IgE, and eosinophil count within three months of the initial reaction. For analysis, patients were classified into two groups based on whether they developed tolerance to baked forms of the offending food before the age of two years. Patients were classified as baked food-tolerant if they had a negative OFC test to a baked form of the allergenic food or if they consumed a baked form of the allergen in an amount equivalent to an OFC every day for at least a week without any symptoms. The baked food-reactive group consisted of patients who, after the age of two, had either a positive oral food challenge (OFC) under the supervision of a physician or a documented history of an immediate reaction following the accidental ingestion of baked trigger food containing protein amounts equal to or less than those used in the OFC.

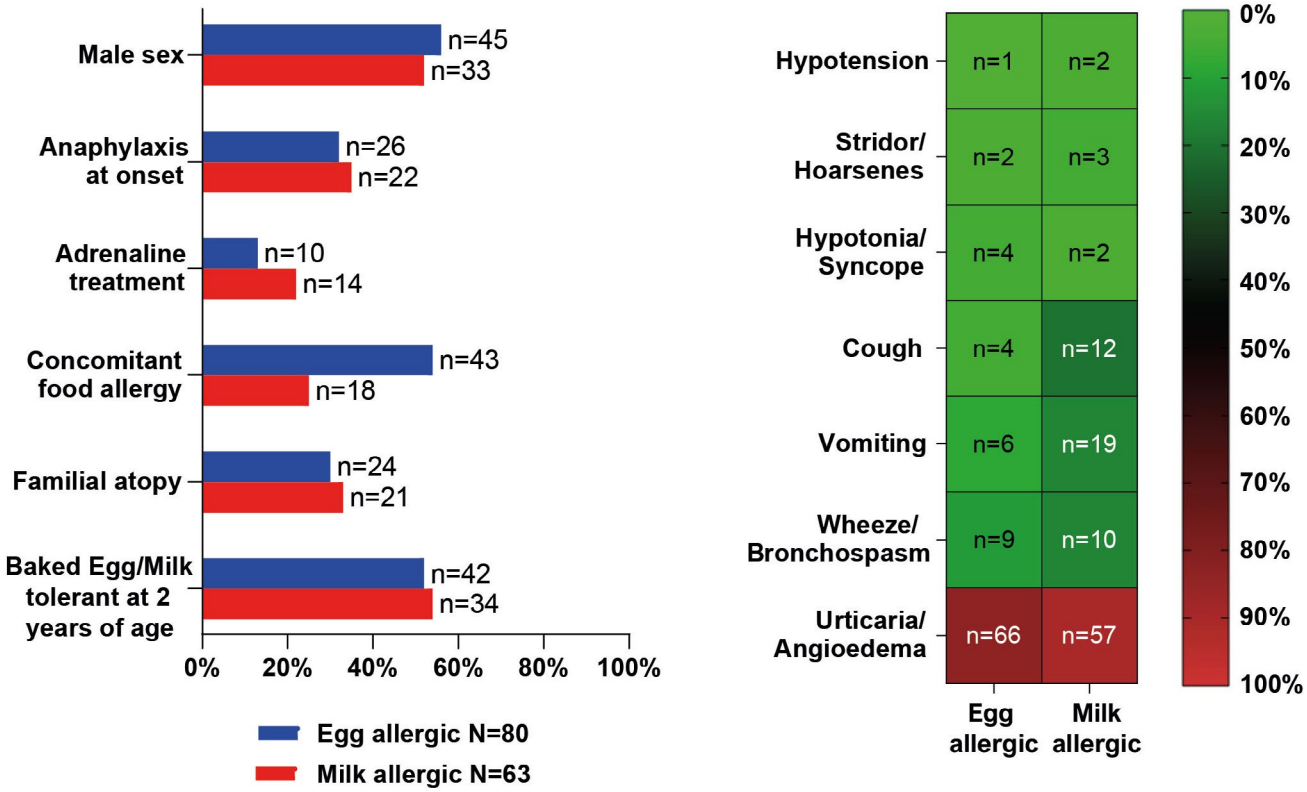
The OFC was conducted as an open challenge under physician supervision using the recipes formulated by the Jaffe Institute of

Food Allergy (New York) (36) for both BM and BE challenges. This involved administering a cumulative dose of 1.6 grams of milk protein for milk allergy and a cumulative dose of 2 grams of egg (yolk and white) protein for egg allergy.

Statistics

For the comparison between the baked food-tolerant and baked food-reactive groups, we employed the χ^2 test for categorical variables and the Mann-Whitney U test for continuous variables. The potential prognostic factors analyzed included sex, family history of atopy, age at the initial reaction, severity of the initial reaction (dichotomized as anaphylaxis or not), presence of concomitant food allergy at presentation, total IgE levels, eosinophil count, and allergen-specific IgE levels at the time of the first reaction. The effect of each prognostic factor was examined in a univariate analysis. P-value < 0.05 was considered to indicate a statistically significant result. The risk factors for BE/BM tolerance were determined by logistic regression analysis. For milk and egg allergies, logistic regression analysis was conducted using variables that were statistically significant in univariate analysis in terms of tolerance/reactivity and those that were not statistically significant but could be clinically meaningful with p < 0.2. We decided to include five variables for egg-allergic patients and six variables for milk-allergic patients in the potentially multivariable logistic regression model. The contributions of each variable included in the model using the hierarchical block method were evaluated with the Akaike information criterion (AIC), Bayesian information criterion (BIC), and Nagelkerke R^2. For milk-allergic patients, the variables of sex and milk-sIgE levels, and for HE-allergic patients, eosinophil count, were excluded from the models, as they did not contribute significantly to the model fit. Regarding multicollinearity, all variables were assessed using correlation analyses, VIF, and tolerance values. Residual and Cook's distance values were checked. Although there were a few cases with residual values > 3, none of them had Cook's distance > 1, so no case was removed from the dataset. Ultimately, 4 variables (female sex, no family history of atopy, no anaphylaxis at onset, and HE white-sIgE level) were included in the model for the egg-allergic group; while 4 variables (no family history of atopy, not anaphylaxis at onset, no concomitant food allergy at onset, casein-sIgE

Figure 1 *- Demographic and clinical characteristics of patients with egg and milk allergies at presentation.*



(A) Demographic and clinical characteristics are shown; **(B)** Symptoms and signs during the initial reaction are shown. Data are presented as numbers and percentages. n: number.

level) were included for the cases with milk allergy. The omnibus test confirmed the models' overall fit ($p < 0.01$). Receiver operating characteristic curve (ROC) analysis was used to determine initial sIgE levels that distinguished patients with BE/BM reactive and tolerant with the highest sensitivity and specificity. Statistical analysis was conducted by Jamovi 2.3.26 version (The Jamovi Project, Australia) and graphs are produced by GraphPad Prism 9 (GraphPad Software Inc., San Diego, California).

Results

Among the 143 patients enrolled, 78 were male and 65 were female; 80 were allergic to egg, and 63 were allergic to milk. The median (interquartile range, IQR 25%-75%) age at onset and at the last visit for participants was 6 (3-6) months and 45 (32-72) months for egg allergy, and 5 (3-6) months and 48 (36-76) months for milk allergy, respectively. The demographic and clinical characteristics of the patients are presented in **figure 1** and **table I**. Among the 80 patients with egg allergy, 41 (51%) had concomitant milk allergy, 1 (2%) had peanut allergy, and 1 (2%) had tree nut allergy. Among the 63 patients with milk allergy, 18 (29%) had concomitant egg allergy.

Seventy-six patients (53%) were found to be baked-food tolerant by the age of two years; 52 (68%) were confirmed with a negative open OFC test, and 24 (32%) were confirmed based on a history of tolerating baked food at home without any reactions. Sixty-seven patients (47%) had ongoing reactivity to baked food after two years; this reactivity was confirmed in 43 (64%) with a positive open OFC and in 24 (36%) with a convincing history of an immediate type reaction after reintroduction of baked food at home. The demographic, clinical, and laboratory features of the study group at the time of the first reaction were comparatively analyzed between the baked-food tolerant and reactive groups for both milk-allergic and egg-allergic children (**table II** and **figure 2**). The fitness of the multivariate logistic regression model for egg-allergic patients, which included four variables (female sex, no family history of atopy, no anaphylaxis at onset, and egg white-sIgE level), was confirmed by the omnibus test ($p < 0.001$). The model explained 33% of the variance (Nagelkerke $R^2 = 0.330$). In the multivariate logistic regression model, no anaphylaxis at onset was found to be an independent predictor of tolerance to baked egg. According to the likelihood ratio (LR) analysis, no anaphylaxis at onset and male sex contributed the most to the

Table I *- Demographic and clinical characteristics of patients with egg and milk allergies.*

	All (n = 143) (100%)	**Egg allergy (n = 80) (56%)**	**Milk allergy (n = 63) (45%)**
Sex n (%)			
Male	78 (54.5%)	45 (56%)	33 (52%)
Female	65 (44.5%)	35 (44%)	30 (48%)
Age at last visit (mo) median (IQR 25%-75%)	46 (33-73)	45 (32-72)	48 (36-76)
Age at onset (mo) median (IQR 25%-75%)	5 (3-6)	6 (3-6.3)	5 (3-6)
Age at diagnosis (mo) median (IQR 25%-75%)	6 (5-8.5)	6.8 (5-9)	6 (4-8)
Reaction at onset			
No anaphylaxis n (%)	95 (66%)	54 (68%)	41 (65%)
Anaphylaxis n (%)	48 (34%)	26 (32%)	22 (35%)
Use of adrenaline	24(17%)	10 (13%)	14 (22%)
Use of antihistamines/steroids	24(17%)	16 (20%)	8 (13%)
Concomitant food allergy n (%)*	61 (43%)	43 (54%)	18 (25%)
Family atopy n (%)	45 (31%)	24 (30%)	21 (33%)
Outcome for baked food consumption by the age of 2			
Tolerant n (%)	76 (53%)	42 (52%)	34 (54%)
Proven by OFC	52 (68%)	31 (66%)	22 (65%)
Safely reintroduced at home	24 (32%)	10 (24%)	12 (35%)
Reactive n (%)	67 (47%)	38 (48%)	29 (46%)
Proven by OFC	43 (64%)	27 (71%)	16 (55%)
Safely reintroduced at home	24 (36%)	11 (29%)	13 (45%)

IQR: interquartile range; mo: months; n: number; OFC: oral food challenge. *Represents concomitant egg allergy for milk allergic patients and concomitant milk allergy for egg allergic patients.

Table II *- Predictive factors at infancy for the tolerance development for baked egg (BE) and/or baked milk (BM) at the age of two years.*

Predictors*	Egg allergy (n = 80) (100%)			Milk allergy (n = 63) (100%)		
	BE tolerant n = 42	BE reactive n = 38	P-value	BM tolerant n = 34	BM reactive n = 29	P-value
Sex						
Male Female	18 (43%) 24 (57%)	27 (71%) 11 (29%)	**0.011**	14 (41%) 20 (59%)	19 (65%) 10 (35%)	0.054
Age at onset (mo)	5.5 (3-7)	6(3-6)	0.763	5 (3-6)	6 (3-6)	0.606
Anaphylaxis at onset						
No Yes	36 (86%) 6 (14%)	18 (47%) 20 (53%)	**< 0.01**	29 (85%) 5 (15%)	12 (41%) 17 (59%)	**< 0.001**
Familial atopy						
No Yes	34 (80%) 8 (20%)	22 (58%) 16 (42%)	**0.025**	28 (74%) 6 (26%)	14 (48%) 15(52%)	**0.004**
**Concomitant Food allergy						
No Yes	17 (40%) 25 (60%)	20 (53%) 18 (47%)	0.268	28 (82%) 6 (18%)	17 (65%) 12 (35%)	**0.038**
Eosinophil count/mm³	355 (225-613)	625 (300-960)	0.055	475 (300-700)	400 (200-800)	0.934
Total IgE (IU/ml)	75 (27-135)	87 (34-216)	0.350	83 (45-142)	79 (33-204)	0.874
Egg white-sIgE (kU/L)	5.1 (2.4-18.6)	12.1 (6.4-44.2)	**0.002**			
Milk-sIgE (kU/L)				4.9 (3.1-9.3)	8.8 (6-18.5)	**0.003**
Casein-sIgE (kU/L)				3.3 (1.6-6.1)	8.2 (4.9-17.2)	**< 0.001**

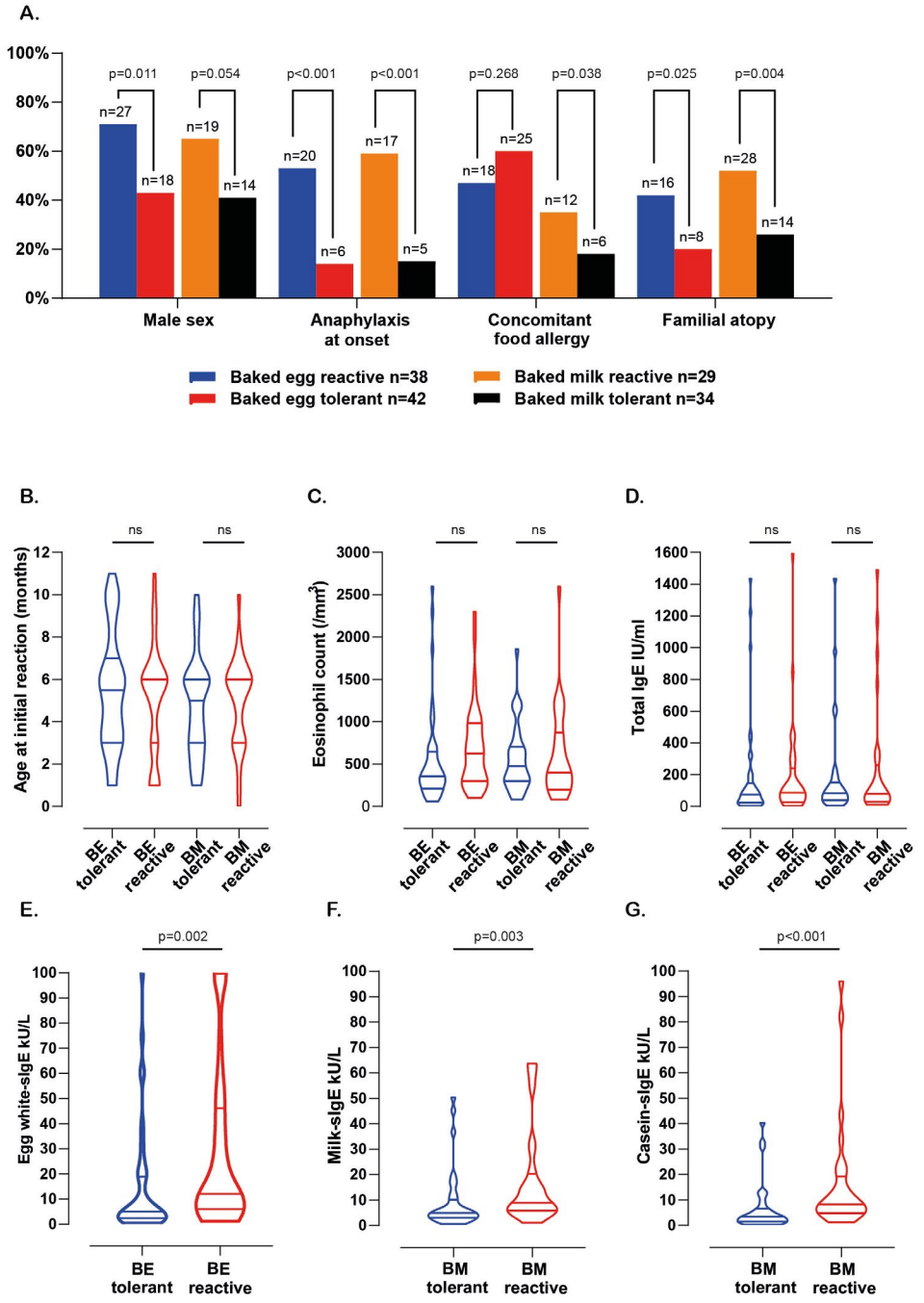
*Data presented with n (%) for categorical data and median (IQR 25-75%) for non-parametric continuous data Statistical comparisons were made by χ^2 test for categorical variables and Mann-Whitney U test for continuous variables. **Represents concomitant egg allergy for milk allergic patients and concomitant milk allergy for egg allergic patients; BE: baked egg; BM: baked milk: IgE: immunoglobulin E; IQR: interquartile range; mo: months; n: number; sIgE: specific IgE.

model, while egg-sIgE contributed the least (LR test χ^2 values 7.94, 3.95, and 1.79; p = 0.005, p = 0.047, and p = 0.180, respectively). The results of the logistic regression analysis for egg allergic patients are shown in **table III**. The model demonstrated an AUC of 0.788 with a sensitivity of 60.5%, a specificity of 60.5%, and an accuracy of 73.8%, respectively.

The fitness of the multivariate logistic regression model for milk allergy cases including 4 variables (no family history of atopy, no anaphylaxis at onset, no concomitant food allergy at onset, casein-sIgE level) was confirmed by omnibus test (p < 0.001). The model explained 49% of the variance (Nagelkerke R^2 = 0.487). In the multivariable logistic regression model, no anaphylaxis at onset, no family history of atopy, and no concomitant food allergy were found to be independent predictors of tolerance to baked milk. According to LR analysis, no anaphylaxis at onset and no family history of atopy contributed the most to the model, while casein-sIgE level contributed the least (LR test X^2 values 12.6, 7.6, 2.9, p < 0.01, p = 0.06, p = 0.089, respectively). The results of the logistic regression analysis for Milk allergy cases are shown in **table III**. The model demonstrated an AUC of 0.859 with a sensitivity of 79.4%, a specificity of 72.45%, and an accuracy of 76.2%, respectively.

The optimal sIgE cut-offs predicting baked allergenic tolerance from the time of initial reaction at the onset by ROC analysis was 7.39 kU/L for egg white-sIgE, 5.99 kU/L for milk-sIgE and 4.02 kU/L for casein-sIgE. Furthermore, we identified cut-off points with 100% positive predictive value for egg white-sIgE and 100% positive and 100% negative predictive value for milk-

Figure 2 *- Comparison of initial characteristics of egg/milk allergic patients with and without tolerance to baked allergen in the first 2 years of life.*



(A) Demographic, clinical characteristics at presentation. Data are presented as numbers and percentages, and the statistical comparison of the baked egg/milk tolerant *vs* reactive group was made by using χ^2 test; significance was set at $p < 0.005$; **(B)** Age at onset; **(C)** Eosinophils counts; **(D)** Total IgE levels; **(E)** Egg white-sIgE levels; **(F)** Milk-sIgE levels; **(G)** Casein-sIgE levels. **(B-G)** Data are presented as median (IQR 25-75%), and the statistical comparisons of baked egg/milk tolerant *vs* reactive groups were made by Mann-Whitney U test; significance was set to $p < 0.05$. BE: baked egg; BM: baked milk; IgE: immunoglobulin E; IQR: interquartile range; n: number; ns: not significant; sIgE: specific IgE.

Table III - *Predictors for early tolerance at age two by logistic regression analysis of baked eggs or milk allergies.*

	Predictor	Estimate	Wald	P-value	OR (95%CI)
Egg allergy	Intercept	-1.9320	-2.48	0.013	0.145 (0.0315-0.667)
	Female gender	1.0362	1.95	0.051	2.818 (0.9959-7.976)
	No familial atopy	1.0374	1.72	0.085	2.822 (0.8651-9.205)
	No anaphylaxis at onset	1.5961	2.71	**0.007**	4.934 (1.5542-15.662)
	Egg white-sIgE value	-0.0139	-1.32	0.187	0.986 (0.9660-1.007)
Milk allergy	Intercept	-3.5128	-2.80	0.005	0.0298 (0.00255-0.348)
	No familial atopy	1.9795	2.55	**0.011**	7.2392 (1.57688-33.234)
	No anaphylaxis at onset	2.4948	3.13	**0.002**	12.1198 (2.54524-57.711)
	*No concomitant allergy at onset	1.6040	1.97	**0.049**	4.9729 (1.00655-24.569)
	Casein-sIgE value	-0.0595	-1.63	0.103	0.9422 (0.87724-1.012)

The statistical analysis employed the binomial logistic regression. Estimates represent the log odds of "baked tolerant" *vs* "baked reactive". OR: odds ratio; CI: confidence intervals; IgE: immunoglobulin E; sIgE: specific IgE. *Represents concomitant egg allergy for milk allergic patients.

sIgE and casein-sIgE as shown in **table IV**. Furthermore, the optimal sIgE cut-offs predicting baked food-reactive from the time of initial reaction at the onset by ROC analysis was 7.39 kU/L for egg white-sIgE 5.99 kU/L for milk-sIgE and 4.02 kU/L for casein-sIgE.

Discussion and conclusions

The current study evaluated the outcomes of patients with IgE-mediated allergies to egg and milk that began in the first year of life, specifically focusing on their tolerability of baked food by the age

Table IV - *Specific IgE cut-off values indicate the outcome being tolerant to baked milk/ baked eggs by ROC analysis.*

		Cut-off (kU/L)	AUC	Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	Accuracy (%) (95%CI)
Egg white-sIgE	Optimal	≤ 7.39	0.703	67 50-80	74 57-80	67 56-77	74 57-80	70 59-80
	100% PPV	≤ 1.2	0.703	12 4-26	100 91-100	100	51 48-53	54 42-65
Milk-sIgE	Optimal	≤ 5.99	0.716	65 47-82	76 57-90	76 61-86	65 53-75	70 57-81
	100% PPV	≤ 1	0.716	6 1-19	100 88-100	100	48 45-50	49 36-62
	100% NPV	≤ 54	0.716	100	14 4-32	58 54-61	100	60 47-72
Casein-sIgE	Optimal	≤ 4.02	0.749	62 44-78	86 68-96	84 67-93	66 55-75	73 60-83
	100% PPV	≤ 1.2	0.749	21 9-38	100 88-100	100	52 48-56	57 44-70
	100% NPV	≤ 43.4	0.749	100 90-100	10 2-27	57 54-60	100	59 46-71

AUC: area under curve; CI: confidence intervals; IgE: immunoglobulin E; NPV: negative predictive value; PPV: positive predictive value; ROC: Receiver Operating Characteristic; sIgE: specific IgE.

of two years. Given that egg and milk allergies are the most prevalent food allergies and that the co-existence of these two allergens is common in our population, we concentrated our attention on these clinically significant allergens.

For patients with egg allergy, using continued BE reactivity beyond age two as a reference, BE tolerability before age two years was linked to female sex, absence of anaphylaxis at presentation, no family history of atopy, and lower egg white-sIgE levels. However, logistic regression analysis revealed that the absence of anaphylaxis during the initial reaction was the only independent predictor of BE tolerability, increasing the likelihood of tolerance before two years by fivefold.

Similar analyses for patients with milk allergy showed that BM tolerability before age two was associated with the absence of anaphylaxis during the initial reaction, no family history of atopy, absence of concomitant egg allergy, low baseline milk-sIgE, and casein-sIgE levels. In a multivariate analysis, we found that no anaphylaxis as the initial reaction, no familial atopy, and no concomitant egg allergy were independent predictors of tolerability to BM before age two.

Previous studies from other groups have investigated factors associated with BE/BM tolerability at an early age in milk- and egg-allergic children. Cogurlu *et al*. (11) reported that male sex was the sole risk factor for BM reactivity, while multiple food allergies and urticaria as the first reaction were independent factors for BE reactivity. Sirin Kose *et al*. (13) found that gender, age, and the nature of symptoms at presentation were not risk factors for BE and BM reactivity at a later point.

We propose that the eligibility criteria in different studies, including the current one, may be an important factor in explaining the heterogeneous results. Our study included children with IgE-mediated immediate-type food allergy that began in the first year of age and excluded those patients with atopic dermatitis. These criteria differed from the other studies. Another potential factor could be the rate of anaphylaxis at presentation, which was higher in our study.

In a recent study examining a similar population with immediate-type reactions before the age of one year, anaphylactic episodes, atopic dermatitis before the age of one year, and high egg white-sIgE levels were identified as poor prognostic factors for egg allergy. Similarly, for milk allergy, anaphylactic episodes, concomitant egg allergy, atopic dermatitis before the age of one year, and high milk-sIgE levels were found to be poor prognostic factors. While the results of our study align with these findings, the discrepancies may be attributed to differences in study design. The previous study did not report on familial atopy (35), whereas our study excluded patients with moderate to severe atopic dermatitis. While there is no consensus on the prognostic factors of IgE-mediated egg and milk allergy (6, 37-44), numerous factors have been identified that vary depending on the population, age group of patients, and clinical characteristics of the examined patients. These factors for egg allergy include onset age, the initial symptom being a systemic reaction, accompanying asthma, atopic dermatitis (7, 45), high sIgE levels in the first reaction, family history of atopy (46), and the size of the skin prick wheal (47). History of systemic reactions (48, 49), and comorbidities such as asthma and allergic rhinitis (48) are risk factors for persistent milk allergy. In addition to these known facts, we think that we have presented an important contribution by elucidating the prognostic factors for food allergy tolerance *vs* persistence at the age of 2 depending on the data-driven at the time of the first reaction in a well-defined, younger and more specific group of patients with milk and/or egg allergy. This information will assist physicians in the evaluation of similar patients in the primary care setting in determining, at the time of diagnosis, which patients should be referred to reference centers for treatment, such as immunotherapy, if necessary, and which patients can be safely followed up in terms of tolerance development.

Efforts to find a reliable clinical or laboratory predictor to determine BE/BM tolerance have been ongoing. Among the tests, it has been suggested that egg white-sIgE is one of the laboratory parameters that best predicts BE tolerance (31, 50, 51), but some claim otherwise (41, 52). Although it has often been suggested that milk-sIgE and casein-sIgE levels are higher in BM-reactive patients than in BM-tolerant (14, 32, 36, 53), there are also studies suggesting that there is no difference in milk-sIgE or casein-sIgE (11, 29). Studies investigating the cut-off value of egg white-sIgE that distinguishes BE-tolerant from BE-reactive subjects have generally examined final values just before OFC testing (31, 50-52, 54). The sIgE cut-off values to differentiate between BM-tolerant and reactive groups have been investigated mainly in patients > 2 years of age and different values have been found (12, 53, 55). Studies reporting initial sIgE cut-off values have aimed to distinguish between those whose allergy resolves and those whose allergy persists. Nevertheless, the threshold for the baseline sIgE level varies according to the study design, including whether it is population-based, single-center, or tertiary center, as well as the clinical characteristics of the participants (37, 56, 57). On the other hand, in cohort studies of patients with egg and milk allergy, especially those with immediate-type reactions, although baseline sIgE levels were higher in the persistent group than in the tolerant group, no discriminative cut-off value was determined (46, 58). Although our results support these studies, we have additionally determined egg white-sIgE, milk-sIgE and casein-sIgE cut-off values as both optimal and 100% positive predictive values, distinguishing the BE/BM tolerant group from the reactive group. However, these values are not sufficient to replace OFC yet. It is crucial to note that the sIgE cut-offs are specific to our population and may vary in other populations. Therefore, it is essential that each center establishes its own sIgE cut-offs for its respective area or population, taking into account the methodology employed for sIgE detection.

One of the limitations of our study is its retrospective design. However, we attempted to mitigate this limitation by excluding patients with incomplete data or follow-up. Another limitation is that our study may not reflect the general population, given that it was conducted in a tertiary clinic where severe cases are often referred. Despite these limitations, the study's key strengths lie in its inclusion of a unique cohort of patients diagnosed during infancy, particularly those with IgE-mediated rapid-type reactions, and its analysis of the initial laboratory tests conducted on all patients during their first year of life.

In conclusion, physicians need guidelines to predict whether young infants with egg or milk allergies will tolerate baked forms of these foods later in life. This study provides valuable insights into the management strategies of food-allergic children. We propose that the identified risk factors could help determine which patients might be candidates for an OFC with baked foods within the specified age periods and who should be referred to a specialized center for future management.

Fundings

None.

Contributions

EK-A, MYA: conceptualization, supervision. MYA, EYG, SC, RA, NO, SeBo, SBE, SaBa, AO, EK-A methodology, data curation. MYA, EK-A: formal analysis. MYA, E.K-A, AO: writing – original draft. All authors: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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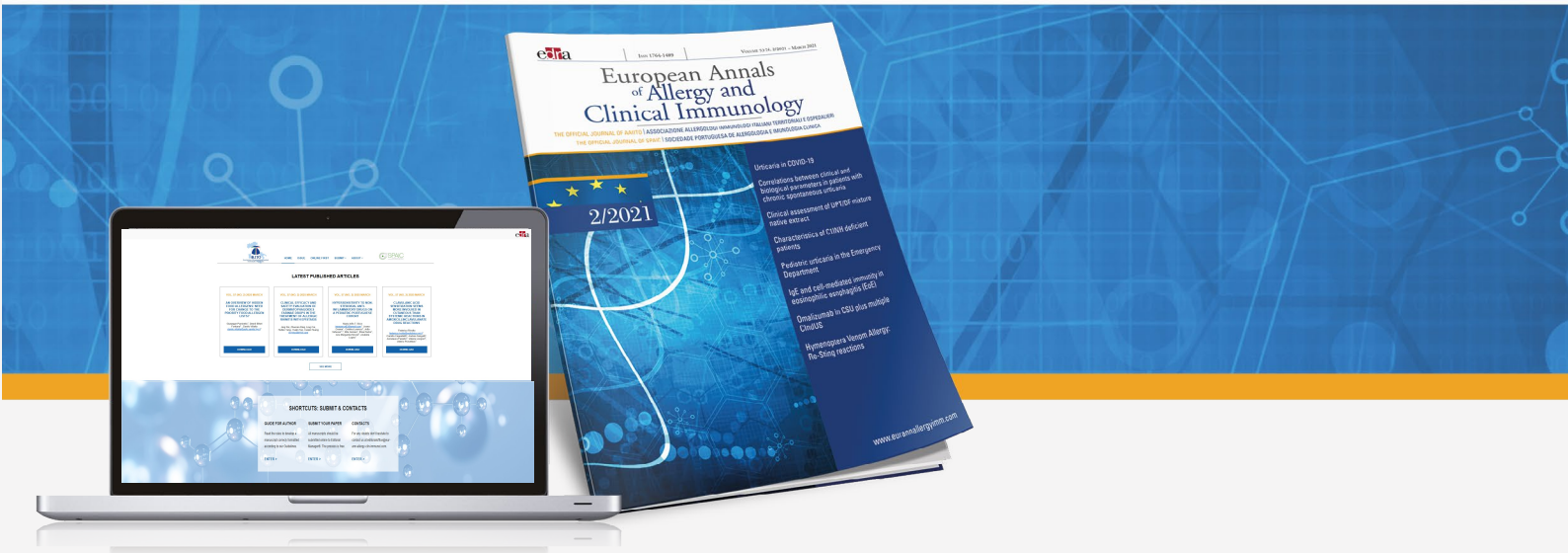
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