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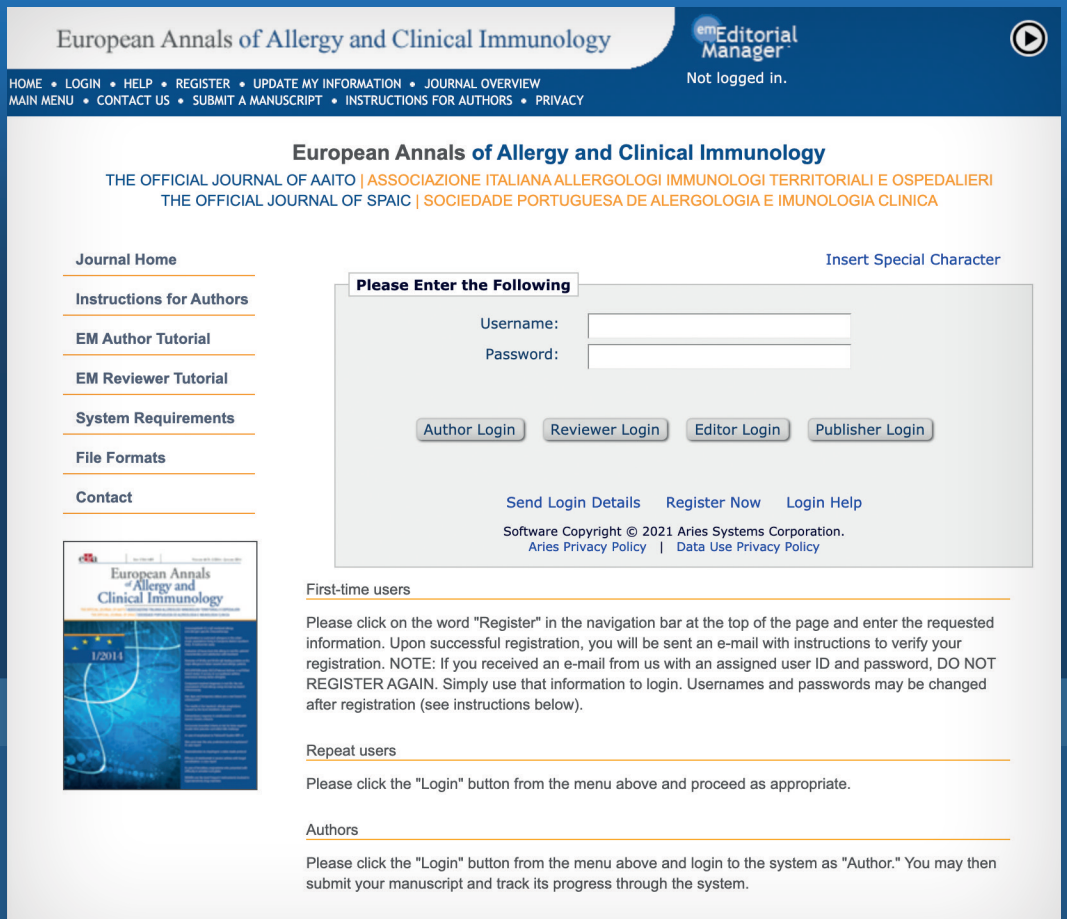
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EDRA SpA

Via G. Spadolini, 7
20141 Milano - Italy
Tel. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
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REVIEW | Eur Ann Allergy Clin Immunol | Vol 56, N.6, 243-251, 2024

Alexander Pampura[1,2], Natalia Esakova[2], Sergey Zimin[1], Evgenia Filippova[2]

Anaphylaxis biomarkers: present and future

[1]Morozov Children's City Hospital, Moscow, Russian Federation
[2]Veltischev Research and Clinical Institute for Pediatrics and Pediatric Surgery of the Pirogov Russian National Research Medical University of the Russian Ministry of Health, Moscow, Russian Federation

Key words

Biomarkers; anaphylaxis; allergy; predictors; tryptase.

Corresponding author
Natalia Esakova
Veltischev Research and Clinical Institute for Pediatrics and Pediatric Surgery of the Pirogov Russian National Research Medical University of the Russian Ministry of Health
Taldomskaya Street
2125412 Moscow, Russian Federation
ORCID: 0000-0001-8792-2670
E-mail: env007@rambler.ru

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Summary

Anaphylaxis is a severe, rapidly developing, and life-threatening systemic hypersensitivity reaction. The diagnosis of anaphylaxis is primarily clinical. Numerous studies on the mechanisms and the biomarkers of the disease are initiated every year. The biomarkers of anaphylaxis may become an important tool for the diagnosis, prevention, repeated risk assessment, severity stratification, and new therapeutic strategies for treatment of the disease. Various immune and non-immune mediators produced and released by effector cell populations are currently considered as biomarkers of anaphylaxis. Here, we review the current data on potential biomarkers of anaphylaxis and the possibilities and perspectives for their use in future clinical practice.

Impact statement

Specific, nonspecific and genetic biomarkers of anaphylaxis have been characterized. In addition to tryptase and sIgE, promising and practically applicable new biomarkers of AF are chymase, carboxypeptidase A3, CCL2 and basogranulin. Genetic biomarkers can predict AF severity and identify high-risk patients.

Introduction

Anaphylaxis (AF) is a severe, rapidly developing, and life-threatening systemic hypersensitivity reaction (1-3). The diagnosis of AF continues to be primarily clinical and is verified by one or more clinical systemic criteria of AF (1-3). The search for AF biomarkers remains one of the most relevant research areas in the AF field, since these biomarkers may reflect the pathogenetic mechanism of AF, allow to confirm the diagnosis of AF, evaluate the need for epinephrine injection, and predict the risk of AF development, severe course, and fatal outcome (4-7).

AF poses several features for the search for biomarkers and their practical use. The involvement of body systems (respiratory, cardiovascular system, gastrointestinal tract, skin, central nervous system) in the AF varies significantly, and symptoms can range widely. For example, skin lesions may be absent or delayed, be first sign of the AF, vary from moderate erythema to giant urticaria and life-threatening angioedema. The existing diagnostic criteria for AF are not always unambiguous, especially for mild and moderate disease, complicating the timely identification of this group of patients. A patient with AF may have a varying pattern of symptoms and threshold level of trigger exposure, exposure to cofactors, *etc*. When provoked by the same trigger in the same patient, the symptoms may also vary. The concentration of potential biomarkers in the body fluids varies depending on the stage of AF, the profile and rapidity of the development

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of the symptoms (first minutes or hours after contact with the allergen or delayed reactions), the exposure to the trigger, and many other factors. This leads to obvious problems in standardizing the conditions for obtaining the biological material and interpreting the test results in wide clinical practice. In addition, the busy emergency environment limits the scope of a diagnostic workout. It is extremely difficult to find a universal and practically applicable AF biomarker. Given the various underlying mechanisms of the AF, measuring only one biomarker may not be sufficient to verify the diagnosis.

Biomarker is defined primarily as the quantitative data of physical exam and/or laboratory parameters with the defined "normal" and "pathologic" limits (8). The optimal biomarker should be highly specific, sensitive, predictive, fast, easy to use, affordable, and as non-invasive as possible. Many cell populations participate in the development of AF, with the largest role of mast cells and basophils: though other cell populations may dominate in various endotypes of AF. AF biomarkers include specific immunoglobulins of class E (sIgE), key actors in the development of immune IgE-mediated AF, and several nonspecific biomarkers (histamine, lipid mediators of inflammation, chemokines and cytokines, proteases, *etc*.), produced and released by various populations of effector cells and involved in the pathogenesis of immune and non-immune systemic reactions. In addition, genetic AF biomarkers have several advantages and a high potential to group patients with a high risk of AF and its severe course, and are currently being actively studied and identified.

Materials and methods

To discern anaphylaxis biomarker studies for this review, we conducted a literature search in August 2023 using PubMed, Scopus, eLIBRARY databases. The keywords used in the search were: anaphylaxis biomarkers, biomarkers of anaphylaxis severity, plasma/serum anaphylaxis biomarker, genetic anaphylaxis biomarkers, histamine in anaphylaxis, tryptase in anaphylaxis, carboxypeptidase in anaphylaxis, neutrophil anaphylaxis biomarkers, platelet activation factor in anaphylaxis, angiotensin converting enzyme in anaphylaxis, heparin in anaphylaxis, cytokines in anaphylaxis, mastocytosis in anaphylaxis. Based on the search more than 6,000 articles were retrieved. Articles with the following characteristics were selected for final analysis: original research investigating specifically the contribution of various biomarkers to the pathogenesis of AF and its severity for the period 1990-2023; studies conducted using cell cultures, animal models, or human subjects. Articles with the following characteristics were rejected: articles not relevant to the study of the role of various biomarkers in the pathogenesis of AF and its severity, period up to 1990, conference abstracts, letters or commentary, editorial and opinion articles lacking original data; articles written in a language other than English or Russian. The search was executed by two authors (AP and NE), using both databases and the keywords listed. The inclusion of an article was based on consensus by both authors. If no consensus could be obtained, the opinions of other authors were sought after (EF and SZ) to determine the outcome of the article. Based on the search and selection process, 58 studies on various anaphylaxis biomarkers were final selected and analyzed for this review.

Results and discussion

Specific class E immunoglobulins

The mechanism of IgE-mediated AF is based on a typical allergic immediate-type reaction caused by the interaction of sIgE antibodies with relevant allergens. Serum sIgE testing in suspected AF to identify possible clinically significant sensitization and the trigger of systemic reaction is an important and integral stage of allergic examination. Specific IgE level is evaluated against allergen sources (*i.e*., walnut, cashew, egg white, egg yolk, *etc*.) and/or molecular components (preferably recombinant ones). The listed tests are useful mostly in diagnosing food AF, and also in drug, insect, and other types of AF (food-dependent exercise-induced, idiopathic) (4, 9, 10). sIgE to the relevant allergen is found in almost all children with food AF induced by a specific food product. We observed only a few patients with food AF with reference level of specific IgE to the relevant allergen, but as a rule, over time, the level of specific IgE is determined above normal. The concentration of the biomarker in children with AF varies significantly and does not always correlate with the severity of the reactions (11, 12). The high sensitization (> 100 kU/L) to fish/seafood was associated with inhalation hypersensitivity to the allergen and determined the elimination measures (excluding being in a room where fish/seafood is fried, boiled, or butchered) to prevent repeated AF in this group of patients (12, 13). Specific IgE for molecular components of the allergen are relevant for identifying high-risk patients for the development of systemic (including cross, and severe) reactions or tolerance to the allergen. High sensitization to milk casein (Bos d 8) (17), to egg white ovomucoid (Gal d1) (18) and sensitivity to recombinant peanut allergens (Aga h1, Aga h2, Aha h3, Aha h6, Aha h9) (19, 21) are predictors of severe and systemic reactions, as well as markers of a long period of development of tolerance or its absence. Garib *et al*. found sIgE to at least one casein, especially to α-casein, in all children with severe (grade 4-5) AF caused by cow milk proteins. In patients with less severe AF (grade 1-3), only 12 out of 21 patients were found to have sIgE to caseins (21). Sensitization to omega-5-gliadin is specific in wheat-dependent physically induced AF, it was found in 80-90% of the patients, whereas sIgE to wheat flour extract was found only in 20-30% of the patients (4). Sensitization to nonspecific lipid transporter proteins (nLTPs) of different plant food allergens (*e.g*., peach (Pru p3), apple (Mal d 3) and apricot

(Pru ar 3), *etc*.) may determine not only a high cross-allergy between different plant species, but due to the physicochemical properties of the allergen (resistance to heating and hydrolysis) a higher risk of systemic reactions. In patients with a history of AF, a significant decrease in sIgE to a causally significant food allergen and its specific components allows introducing the product by conducting a provocative test.

Specific IgE are undoubtedly the key biomarkers of IgE-mediated AF. They are used for differential diagnostics, diagnostics, identification of the trigger of a systemic reaction, determination of the overall risk and severity of AF, prognosis of the tolerance development to the allergen, and evaluation of the possibility of introducing of causally significant product into the patient's diet/life. However, various questions remain about the relationship between IgE-mediated sensitization and AF. It is not known why AF develops only in some patients sensitized to a particular allergen, what is the reason for the poor correlation of sIgE concentration with the severity of the systemic reaction, *etc*. These and other clinically significant observations highlight the role of not only sIgE but also of other factors in the development of AF.

Non-specific biomarkers for diagnosis and evaluation of severity of anaphylaxis

Nonspecific AF markers are released by the effector cells (mast cells, basophils, macrophages, neutrophils, *etc*.) during the acute systemic reaction (**table I**). The mediators have different clinical significance (diagnostic value, stratification of the severity, risk of AF, and response to therapy), half-elimination from the body, features of collecting biomaterial for analysis and subsequent interpretation of the results, significantly affecting their use in real clinical practice.

Tryptase is a neutral serine protease contained in the secretory granules of mast cells. Tryptase is produced mainly by mast cells, being a key plasma biomarker of their activation, including the activation during acute AF; basophils can also release tryptase, but in much smaller quantities. Two main isoforms of tryptase are described, α and β. The first one (α-tryptase) is released constantly and increases with an increase in the number of mast cells, as in mastocytosis. Mast cells activation (degranulation) upon AF releases β-tryptase (6). The mast cells of various organs contain a different amount of tryptase, which can determine the dependence of the severity of an acute reaction on the allergen entry route. Commercial tests are available to determine total tryptase (α/β-tryptase), which allows us to evaluate this biomarker in clinical practice. The method of measuring serum tryptase is stable, reliable, and easy, but limited by the timing of biomaterial obtainment. The basal serum tryptase level in healthy people ranges from 1 to 11 ng/ml (ImmunoCAP).

From the first minutes of the AF development, the serum tryptase increases sharply, peaking within 60-90 minutes, followed by a steady decline and disappearance within a few hours (5). If AF is suspected, guidelines recommend blood sampling for the tryptase determination twice: first, in the first 15 minutes to 3 hours after the onset of the first AF symptoms, and second, 24 hours or more after the AF symptoms disappearance to determine the basal level of the marker (1, 2). Measuring the basal level of tryptase outside an acute AF is especially relevant for patients with the tryptase within the reference values, even during the acute systemic reaction. When the tryptase level exceeds the values obtained using the formula *1.2 × baseline + 2 ng/ml*, it can be diagnostically significant for AF (1, 2).

Studies showed the clinical significance of tryptase in AF for diagnosis, evaluation of the risk of development, and stratification

Table I *- Characteristics of nonspecific anaphylaxis biomarkers.*

Biomarker of acute AF	Characteristic	The period of detection from the AF onset / peak time
Tryptase in plasma/serum (1*, 2*, 5, 22-28)	Stable, highly specific for type 1 hypersensitivity. It is used in the diagnosis of AF, with the concentration positively correlating with the plasma/serum histamine and the severity of AF	15 min-3 h/0-90 min **The study of basal tryptase level outside the AF is recommended*
Histamine in plasma/serum (26, 27, 32, 33)	High specificity for type 1 hypersensitivity, correlation with the plasma/serum tryptase and AF severity	5-30 min/5-15 min
Urine histamine metabolites: N-methylhistamine, N-methylimidazole acetate (26, 28, 33)	High correlation with the plasma/serum histamine, correlation with AF severity	Within 24 hours or more/unknown
Chymase in plasma/serum (34, 35, 36)	Potentially stable, may correlate with the plasma/serum tryptase	Within 24 hours or more/unknown
Carboxypeptidase A3 in plasma/serum/saliva (37, 38)	It is found in serum and saliva; limited data on an increase in plasma/serum despite the normal tryptase level	Within 8 hours or more/unknown

→

Biomarker of acute AF	Characteristic	The period of detection from the AF onset / peak time
The major factor of basophil chemotaxis (CCL2) in plasma/serum (39, 40)	May correlate with the AF severity	Within 2 hours/unknown
Neutrophil myeloperoxidase in plasma/serum (41, 42)	Correlates positively with the AF severity	Within 5 hours/unknown
Platelet activation factor (PAF) in plasma/serum (44, 45, 46)	Correlates with the AF severity	3-15 minutes/less than 5 minutes
Platelet Activation Factor-acetyl hydrolase (PAF-AH) in plasma/serum (44, 45, 46)	The concentration of the marker correlates negatively with the severity of AF	Unknown
Angiotensin Converting Enzyme (ACE) (47, 48, 49)	The concentration of the marker correlates negatively with the severity of AF	Unknown
Dipeptidyl Peptidase 1 (DPP1) in plasma/serum (36, 50)	Data are limited, a potential role in the chymase activation	Unknown
Basogranulin in plasma/serum (51)	Data is limited. A unique secretory marker of basophils	Presumably 5-30 min, similar to histamine/unknown
Heparin in plasma/serum (28, 52)	Data is limited. Potential correlation with the AF severity	Unknown
Leukotriene E4 (LTE4) in urine (53)	Data is limited	Unknown
11-beta-prostaglandin F2-alpha in urine (54)	Data is limited	Unknown
Plasma/serum cytokines: interleukins (IL-2, IL-4, IL-5, IL-6, IL-10, IL-13), interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α) (26)	Data is limited. Potential correlation with the AF severity	Within 10 hours/unknown (for some cytokines, 100 minutes)

of the severity; however, the tryptase level depends on several factors (trigger, age of the patient, symptoms). Tryptase increases most often in the insect and medicinal AF, while in the food AF the biomarker in question is less representative and often remains normal, even if blood is sampled at the optimal time. In the perioperative AF in children, the high concentration of blood tryptase is observed in more than half of patients (22, 23); in the drug-caused AF, an increase may be more significant and prolonged (24). Ruëff *et al*. found an increased risk of AF developing during allergen-specific immunotherapy (ASIT) in patients with insect allergy and high basal tryptase (25). Serum tryptase levels positively correlate with histamine concentration, AF severity, severe hypotension, and skin symptoms (erythema and urticaria) (24, 26). In some acute AF patients, the lack of tryptase increase combined with a histamine increase may show the predominant involvement of basophils in the development of a systemic reaction (27). When interpreting the tryptase level, it is important to consider the normal or slightly elevated concentration in 36-40% of acute AF cases, which does not exclude this diagnosis (26, 28). In young children, the basal level of tryptase is elevated initially and reaches the adult norm only by 9-12 months (29), tends to increase with age (30); some data show it is higher in men than in women (31). Tryptase may increase in patients with an acute isolated skin allergic reaction, chronic urticaria, mastocytosis, primary activation syndrome of mast cells, including α-tryptasemia, idiopathic activation syndrome of mast cells and several other conditions not related to AF (oncohematological diseases, helminthiasis, eosinophilic gastrointestinal diseases, chronic renal failure, myocardial infarction) (24).

Tryptase is a recognized factor in pathogenesis, accessible, and routinely used diagnostic biomarker of AF, with a certain predicting ability regarding the severity and risk of AF development. It's challenging to use the marker because of its low sensitivity and blood sampling restrictions.

Histamine is the next important mediator of IgE-mediated allergic reactions, released from granules of mast cells and basophils. Histamine effects most AF symptoms (edema, hyperemia, urticaria, itching, *etc*.) and correlates with the severity of AF (26), but the role of plasma histamine and/or its urine metabolites (N-methylhistamine and N-methylimidazole acetate) as biomarkers of AF remains questionable. Upon the development of AF, the plasma histamine peaks 5 minutes after exposure to the allergen and returns to basal values after 15-30 minutes (32). Therefore, the use of histamine as a nonspecific marker of AF is possible only when collecting blood samples within the first 15 minutes from the start of the reaction, which is extremely difficult. Histamine disappears rapidly from the plasma via methylation by N-methyltransferase to form N-methylhistamine, deaminated by diamine oxidase into N-methylimidazole acetate. The resulting metabolites are stable and can be detected in the urine for 24 hours or more from the onset of AF symp-

toms; some data show their correlation with its severity (28). Commercial kits are available for the study of urine histamine metabolites, however, the use of those kits is not popular. The normal level of these markers does not exclude the diagnosis of AF, and false positive test results are possible, associated with the consumption of histamine-liberators or with systemic mastocytosis (33).

Chymase is a relatively new and still poorly studied biomarker of AF. Chymase is a serine protease found in the secretory granules of mast cells. This marker is quite stable in blood serum and was detected in 8 cases of fatal AF at autopsy; its concentration correlated with the serum tryptase (34). In a similar work, Osawa *et al*. identified chymase in lung mast cells during autopsy of 3 fatal AF cases; the control autopsy cells without AF had no chymase (35). The serum chymase in the patients with food, drug, and insect-caused AF increased compared to control within 8 hours of the first AF symptoms and remained high for at least 24 hours (36).

Carboxypeptidase A3 is a potential mediator of AF released by activated mast cells. Brown *et al*. analyzed the levels of carboxypeptidase A3 in the blood and saliva of 33 patients with suspected drug allergy undergoing provocative tests (37). Baseline basal serum and saliva levels of carboxypeptidase A3 were higher in the patients with positive provocative tests and a history of severe allergic reactions with cardiovascular and/or respiratory symptoms. When analyzing this marker directly at the moment of provocation, its level increased only in the saliva of patients with a positive test. Zhou *et al*. found high levels of carboxypeptidase A3 in plasma/serum samples of patients collected within 8 hours after the onset of a systemic allergic reaction; 70% of these patients had normal tryptase concentration (38). The stability of carboxypeptidase A3, the ability to test it over a wide time range, and the noninvasiveness of testing it in saliva make it a good candidate for further study.

Besides mast cell mediators, C-C motif chemokine ligand 2 (CCL2), the major factor of basophil chemotaxis attracting basophils to the focus of inflammation after exposure to an allergen, is promising. Korosec *et al*. studied the potential of CCL-2 as an AF biomarker in blood samples taken directly during the AF episode, 7, and 30 days after the systemic reaction (39). The study showed the significantly lower absolute number of circulating basophils in the acute AF compared to the non-reaction period. CCL-2 was significantly higher in the acute AF compared with samples taken outside the reaction, with the decrease of CCL-2 to baseline within 2 hours after the onset of AF symptoms. The test had 94% and 96% sensitivity and specificity, respectively. A similar study showed not only a serum CCL-2 increase in the acute systemic reactions, but also its significant positive correlation with their severity (40).

The role of neutrophils in the development of AF has been shown to be activated in the early stages of the reaction, independent of mast cell activation. The main enzyme stored in neutrophils is myeloperoxidase, the level of which in the blood increases at the time of development of clinical symptoms of AF and may correlate with its severity. Francis *et al*. reported that this enzyme was 2.9-5 times higher in patients with severe and moderate AF compared to healthy controls, it remained stable during the first 5 h after the onset of symptoms (41). In an experimental model of AF, an increase in myeloperoxidase levels is detectable 2 minutes after exposure to the trigger (42). There are also reports of increased neutrophil elastase values in the development of drug-induced AF during angiography with ioxaglate (43).

Lipid mediators are synthesized and released from effector cells upon their activation, including that in AF. The role of platelet activation factor (PAF), eicosanoids, and sphingosine-1-phosphate in the AF development has now been proven. Several studies showed a key role of PAF and its cleavage enzyme, platelet activation factor-acetylhydrolase (PAF-AH), in the pathogenesis of AF. PAF is a pro-inflammatory phospholipid synthesized and secreted by mast cells, monocytes, and tissue macrophages. PAF determine the cardiovascular symptoms of AF (hypovolemia, increased vascular permeability, myocardial dysfunction, *etc*.). Series of studies demonstrated high serum PAF and low PAF-AH in an anaphylactic attack, correlating with the severity of AF (44-46) better than tryptase and histamine. Basal PAF-AH in patients with insect-induced AF (outside acute reaction) showed similar results. An important disadvantage of PAF is the extremely short period of life (3-15 minutes) from the AF symptoms development, precluding its clinical use.

Angiotensin converting enzyme (ACE) showed the protective role in the development of life-threatening edema in AF (47, 48). Summers *et al*. noted significantly lowered basal serum ACE in patients with a history of severe swelling of the pharynx and larynx upon the development of acute allergic reactions to food allergens, relative to patients with no such history (49). The relative risk of severe, life-threatening edema of the pharynx and larynx was increased 10-fold upon the decrease in serum ACE concentration in these patients.

Dipeptidyl peptidase 1 (DPP1) is a less studied marker of the acute AF, the level of this peptidase positively correlates with the chymase level, since this enzyme is supposed to activate the chymase (36, 50). Basogranulin, a functionally similar protein of basophil granules, is actively studied as an alternative to histamine serum marker of AF (51). The pathogenesis of anaphylactic reaction involves endothelial cells and various ways of heparin release, activating the complement system (the formation of anaphylatoxins C3a and C5a) and factor XII, the proteolysis of kininogen, and the release of nitric oxide (NO) and bradykinin; the complement activation and the bradykinin level correlate with the severity of AF (28, 52). Urine leukotriene E4 (LTE4) and 11-beta-prostaglandin F2-alpha are of potential significance

in AF (53). The plasma level of several inflammatory mediators, such as IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α), may increase in AF and correlate with its severity, but their sensitivity and specificity as biomarkers has not yet been established (26). Some data show the negative correlation of several cytokines, such as transforming growth factor-β (TGF-β), IFN-γ, and apolipoprotein B (apoB), with the severity of food-caused AF in the pediatric population (54).

Genetic biomarkers of risk and severity of anaphylaxis

Most of the nonspecific acute AF biomarkers described above have significant disadvantages of only relative stability and a limited time frame for biomaterial collecting, which complicates their application and interpretation. Potential genetic biomarkers of AF are sufficiently stable, not time-limited, and valuable outside the acute AF. Therefore, they are potentially useful for the prevention of systemic reactions and the selection of groups of patients with a high risk of severe AF (**table II**). In particular, mutations of the *C-KIT* gene regulating mast cell function were associated not only with mastocytosis but also with idiopathic AF (55). Replication of the *TPSAB1* gene encoding α-tryptase is associated not only with hereditary α-tryptasemia, characterized by an increase in basal tryptase levels, but also with the severity of hymenoptera venom-induced AF (56). Górska *et al*. identified a link between increased tumor necrosis factor receptor-associated factor 4 (*TRAF-4*) gene expression in blood with the development of food-caused AF in patients with mastocytosis (57). Some studies demonstrated the connection of AF with changes in the functioning of genes encoding interleukins, which are crucial in regulating the balance of Th1/Th2 cellular response. Single nucleotide polymorphisms of genes encoding IL4 and IL-10 were detected in patients with AF to penicillin (58, 59). We found a significant decrease in the expression of the platelet activation factor acetylhydrolase gene (*PLA2G7*) in blood of children with food AF (outside the acute episode) compared to the control group without AF (60). The AF severity correlated negatively with the expression of the gene under study, which decreased significantly with the development of AF in patients with a history of cardiovascular symptoms (drop in blood pressure, pronounced tachycardia/bradycardia). We have also obtained interesting data on the angiotensin converting enzyme (*ACE*) gene expression in blood (60). In AF patients, the expression was significantly higher in those with a history of mild, localized, or no edema, compared with those with life-threatening edema of the tongue and soft tissues of the oropharynx.

Signal transducer and activator of transcription 6 (STAT6) is a transcription factor playing a central role in the pathophysiology of allergic inflammation. Sharma *et al*. report rare monoallelic variants of STAT6 in 16 patients suffering from severe and therapy-resistant allergy, with a history of AF in 9 of them (61). Functional studies have established a phenotype with stable phosphorylation of STAT6, increased expression of the target gene STAT6, and a bias towards the Th2 response. According to the authors, the identification of these STAT6 variants should be regarded as a new autosomal dominant allergic disorder.

Recently, anecdotal reports described the role of microRNAs in the molecular mechanisms of AF in humans. MicroRNAs are small non-coding RNAs that regulate gene expression, usually by suppressing transcription. Rodriguez Del Rio *et al*. found in 2021 an increase of several serum micro-RNAs (miR-21-3p and miR-487b-3p) in children upon the development of food AF. The authors proposed its potential in the diagnosis of AF (62). In 2022, Francuzik *et al*. identified hsa-miR-451a in patients with a history of anaphylaxis (63). However, the need to evaluate these markers in dynamics and methodological features of the study (multidirectional changes in tryptase concentration, different triggers, small sample size, *etc*.) show the role of Micro-RNA in the pathogenesis of AF rather than its significance as an objective biomarker of AF.

Table II - *Characteristics of genetic anaphylaxis biomarkers.*

Biomarker	Characteristic
Polymorphism of the *C-KIT* gene (55)	Polymorphism of the gene was detected in mastocytosis and idiopathic AF
Replication of the *TPSAB1* gene (56)	Replication of the *TPSAB1* gene detected in hereditary α-tryptasemia is associated with the severity of hymenoptera venom-induced AF
Expression of the tumor necrosis factor receptor-associated factor 4 (TRAF-4) gene in blood (57)	Increased gene expression is associated with the development of food-caused AF in mastocytosis
Polymorphisms of IL-4 and IL-10 genes (58, 59)	Polymorphism of the gene was detected with drug AF to penicillin
Expression of platelet-activating factor acetylhydrolase (*PLA2G7*) gene in blood (60)	Basal gene expression (outside acute reaction) negatively correlates with the severity of AF
Expression of the angiotensin converting enzyme (*ACE*) gene in blood (60)	The basal gene expression (outside acute reaction) negatively correlates with the frequency of severe AF symptoms

Conclusions

Many studies show that anaphylactic reactions involve a complex and diverse range of immune cells and mediators. The search for new biomarkers of anaphylaxis (AF) is important for verification of diagnosis, its prevention, evaluation of the risk of recurrence and of life-threatening/lethal systemic reactions, stratification of severity, and treatment of AF.

Tryptase is the most useful biomarker for identifying an acute allergic reaction. Measuring sIgE levels to the suspected trigger can help identify high-risk groups for cross- and severe reactions and help with elimination measures. Chymase, carboxypeptidase A3, CCL2, and basogranulin alternative to histamine are the promising new biomarkers for the diagnosis of acute AF, due to their rather long-lasting life span. Further research should show that genetic biomarkers can predict the severity of AF and identify high-risk individuals before AF develops.

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Contributions

AP: conceptualization, writing – original draft, funding aquisition. AP, NE, EF, SZ: writing – review & editing. AP, SZ: supervision.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Leonor Esteves Caldeira[1,#], Rita Limão[1,#], Rita Brás[1], Elisa Pedro[1], Célia Costa[1,2,*,#]

A real-world characterization of a population with eosinophilic esophagitis: looking for severity biomarkers

[1]Department of Immunoallergology, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte EPE, Lisbon, Portugal
[2]University Clinic of Immunoallergology, Medicine Faculty of Lisbon, Lisbon, Portugal
[*]Second corresponding author: dra.celiacosta.73@gmail.com
[#]The authors contributed equally to this work

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Corresponding author
Leonor Esteves Caldeira
Department of Immunoallergology
Hospital Santa Maria
Centro Hospitalar Universitário Lisboa Norte
Avenida Professor Egas Moniz
1649-035 Lisbon, Portugal
ORCID: 0000-0003-2347-4658
E-mail: leonorfsecaldeira@gmail.com

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Impact statement

The presence of atopy and sensitization to airborne and food allergens in EoE patients doesn't seem to be useful for predicting disease severity.

Summary

Background. *Eosinophilic esophagitis (EoE) is an immune-mediated chronic esophageal disease, with frequent association with atopy. A validated non/minimally invasive biomarker of disease severity has not been identified. We aimed to determine if sensitization to airborne and food allergens correlates with disease severity, and to evaluate the association between clinical and laboratory characteristics with the severity of EoE.* ***Methods.*** *Retrospective study of EoE patients observed in a differentiated center, 2009-2021. The association between patients' diagnosis age, disease duration before diagnosis, sensitization to airborne/food allergens, serum total IgE and peripheral blood eosinophil values and severe clinical disease (presence of symptoms with a significant impact on quality of life and/or ≥ 1 hospital admission due to EoE complications, namely severe dysphagia, food impaction or esophageal perforation) and histological severe disease (≥ 55 eos/hpf and/or microabscesses in esophageal biopsies) was evaluated.* ***Results.*** *92 patients were observed, 83% male, 87% atopic. There was a mean delay in diagnosis of 4 years (range 0-31). 84% had aeroallergen sensitization and 71% food sensitization. Food impaction and dysphagia were the most frequent symptoms, and severe clinical disease was observed in 55%. Histologically, 37% had severity criteria. Patients with severe clinical disease had a significantly longer mean disease duration before diagnosis than patients without severe clinical disease (79 vs 15 months; $p = 0.021$). Patients who described food impaction were significantly older at time of diagnosis than those who have never had impaction (18 vs 9 years; $p < 0.001$). There was no significant association ($p < 0.05$) between sensitization, serum total IgE and peripheral blood eosinophil values and clinical or histological severity.* ***Conclusions.*** *An older age at diagnosis and a longer disease duration before diagnosis appear to be useful for predicting EoE clinical severity. Despite having been demonstrated a high prevalence of allergic disease, the presence of sensitization to airborne and/or food allergens do not seem to be useful for predicting clinical or histological severity.*

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Introduction

Eosinophilic esophagitis (EoE), a complex and heterogeneous disease, first described in 1978, is a chronic immune-mediated inflammatory disorder characterized clinically by symptoms of esophageal dysfunction and histologically by an eosinophil-predominant inflammation of the esophagus (1, 2) response to inhalant and food allergens. It is more common in males, with a ratio of 3:1. Although 65% of cases occur during childhood, there is a second peak of incidence between 30 and 44 years of age (3, 4).

Briefly, it is known that the pathogenesis of EoE involves the interaction of genetic and environmental factors leading to impaired epithelial esophageal barrier function, allowing the interaction of environmental and food allergens with the antigen-presenting cells, leading to an inflammatory response mediated by Th2 interleukins (IL), IL-4, IL-5, and IL-13 secreted by Th2 cells, increasing eotaxin 3, recruiting and activating eosinophils, basophils, and mast cells, inducing chronic inflammation, tissue damage, fibrosis and dysmotility with smooth muscle cell constriction (1, 5).

EoE could be triggered by food, mainly milk, often associated with egg, wheat, and soy. It may be associated (15% of cases) with severe IgE-mediated reactions to some foods, mostly peanut, milk, and eggs. Atopic diseases are more prevalent in EoE than in the general population, manifested by asthma, allergic rhinitis, and atopic dermatitis. Besides, recent findings suggest that EoE is a late manifestation of the allergic march in some individuals and that sensitization to foods and/or aeroallergens early in life may predispose to EoE development (6). Nevertheless, the allergic mechanisms remain poorly understood. EoE is associated with a high total Immunoglobulin E (IgE) level and with sensitization to food allergens (75%) and respiratory allergens (73%), but IgE-mediated mechanisms cannot account for all the changes observed and complex mechanisms involving the innate and adaptive immune system may be involved (5, 7).

The presenting symptoms vary with age: in adults the most common are dysphagia, gastroesophageal reflux disease symptoms, heartburn and food impaction, owing to the progressive evolution towards esophageal stenosis. In contrast, children present more commonly with vomiting, heartburn, regurgitation, emesis, and abdominal pain (5, 8). It is known that EoE may negatively affect the quality of life of patients and their families because of symptoms limiting normal feeding, with many patients reporting social and psychological implications of food-related illness (9, 10).

Recent consensus recommendations based on a systematic review of the literature and expert opinion led to the diagnostic criteria that EoE is a clinicopathological disease characterized by 1) esophageal symptoms including but not limited to dysphagia and food impaction in adults and feeding intolerance and gastroesophageal reflux disease (GERD) symptoms in children and 2) eosinophil predominant inflammation of ≥ 15 eosinophils per high power field (eos/hpf) in the esophageal tissue after exclusion of other disorders associated with similar clinical, histologic, or endoscopic features (11). Since eosinophilic infiltration of the esophagus may not be evenly distributed, at least six biopsies should be obtained from the proximal and distal esophagus to maximize the sensitivity (8).

Guidelines for the treatment of EoE were recently revised by the American Gastroenterological Association (AGA) and the Joint Task Force on Allergy-Immunology Practice Parameters (JTF) (12). Swallowed topical corticosteroids are a popular primary therapy for EoE and were the only therapy in the AGA-JTF guideline to receive a strong recommendation based on moderate-quality evidence. Although response to PPIs is lower than reported with most corticosteroid studies, their safety and ease of administration can make them a good first-line treatment option in some patients (12).

Diet therapy is also a first-line option recommended for treatment of EoE in the AGA-JTF guidelines, although considered a conditional recommendation, due to low-quality evidence. Currently available strategies include elemental diets and empirical or target elimination diets. There may be challenges with long-term adherence to dietary elimination and a major limitation to the use these diets in treatment of EoE is the need for repeated endoscopic biopsy assessment during the food reintroduction process (12, 13).

There are some patients who may respond to treatment with endoscopic dilation, which will reduce the risk of future food impaction, but there is no associated benefit in terms of improving histological eosinophilia (10). Whenever possible, treatment should be oriented by a multidisciplinary team, consisting of a gastroenterologist, immunoallergologist and nutritionist for assessment of diet feasibility and nutritional follow-up (12, 13).

Presently, histological examination of esophageal mucosal biopsies is required to establish the diagnosis, assess response to therapy, document disease remission, and evaluate symptom recurrence. The necessity for numerous endoscopies to monitoring the disease and the lack of validated non-invasive invasive biomarkers or tools are the main reasons for the significant burden on patients and on the healthcare system. There is an urgent need for non-invasive or minimally invasive biomarkers. Although, in recent years, several efforts have been tried to identify potential biomarkers for diagnosing, monitoring and predicting severity, and some promising candidates have been described in literature, it continues to be elusive (14).

The aim of this study, that included patients with EoE followed in a Immunoallergology Department of a tertiary Portuguese hospital along 12 years, was to evaluate potential clinical and laboratory biomarkers that could correlate with clinical and histological disease severity and be obtained in a minimally invasive manner,

namely patients' diagnosis age, disease duration before diagnosis, presence of sensitization to airborne and food allergens, serum total IgE and peripheral blood eosinophil values, which could contribute to precision medicine in predicting disease severity.

Materials and methods

Study design and population

A real-world, single-center, retrospective, cross-sectional study was conducted in our Immunoallergology Department in Lisbon, Portugal. We performed an analysis of database of all patients diagnosed with EoE, followed in our department, from 2009 to July 2021. The association between patients' diagnosis age, disease duration before diagnosis, sensitization profile, serum total IgE and peripheral blood eosinophil values and severe clinical disease and severe histological disease were evaluated (the criteria used to define the clinical and histological severity of EoE are defined in sections "Clinical Analysis" and "Laboratory and Endoscopic Analysis", respectively).

The population was characterized according to demographic data, personal history of atopy, defined as personal tendency to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins, according to the World Allergy Organization (15), symptoms associated with the diagnosis of EoE, aeroallergens sensitization and food sensitization by skin prick test (SPT), food atopy patch tests (APT) and specific IgE, according to SPT.

SPT were performed for common aeroallergens with commercial extracts (Bial-Aristegui®, Bilbao, Spain), namely house dust and storage mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Euroglyphus maynei*, *Lepidoglyphus destructor*, *Blomia tropicalis*, *Glycyphagus domesticus*, *Acarus siro*, *Tyrophagus putrescentiae*), dog and cat's fur, *Alternaria alternata*, *Aspergillus fumigatus*, mixtures of grasses, *Phleum pratense*, *platanus*, *Olea europaea*, *Parietaria judaica*, *Artemisia vulgaris* and *Plantago lanceolata*. Patients underwent SPT to selected foods according to clinical history suggestive of IgE-mediated food allergy, in addition to a standard panel of milk and proteins (alfa-lactoalbumin, beta-lactoglobulin, casein), soy, egg (yolk, white, ovalbumin, ovomucoid), meats (beef, chicken, turkey, pork and rabbit), fish (bream, hake, axillary seabream, sea bass, sardine, horse mackerel, cod, tuna, salmon), seafood (shrimp), cereals (rice, wheat, barley, corn, rye, oat), fresh fruits (apple, pear, peach), tree nuts (walnut, hazelnut, cashew, almond, chestnut, pistachio, pinion) and peanut. The SPT were performed by an appropriately trained experienced professional. The positive control was performed using histamine dihydrochloride 10 mg/ml and the negative control was performed using isotonic saline solution. The SPT result was read after 15 minutes by measuring the wheals (mm) using a graduated ruler. Papules with an average diameter measuring 3 mm larger than the negative control was considered positive (16).

The food APT were performed using the following standard fresh foods: cow's milk, egg white (raw and cooked), egg yolk (raw and cooked), wheat flour, cornflour, walnut, hazelnut, cashew, almond, pistachio, pinion, beef, pork, chicken, turkey, shrimp (raw and cooked), hake, codfish and soy. Foods were mixed with isotonic saline and placed in aluminum cups (8-mm Finn Chambers® in scanpor tape, Smartpractice Europe GmBH, Germany) and adhered to the patient's back. The patches were removed at 48 hours and results were read at 72 hours. Reactions considered positive were classified as + for erythema and scattered papules, ++ for erythema and papules, and +++ for erythema and vesicles as per standard patch testing protocols (17).

A written informed consent was obtained from all patients or from the parents or legal guardians of those under 18 years old signed by all patients to use their clinical data in an anonymous form. Patients were treated according to ethical standards established in the Declaration of Helsinki.

Clinical analysis

Severe clinical disease was defined based on our clinical practice as presence of symptoms with a significant impact on quality of life and/or ≥ 1 visit to the Emergency Department and/or hospitalization due to EoE complications, namely severe dysphagia, food impaction and/or esophageal perforation due to the progression of esophageal stricture. In the present study, the burden of disease on quality of life was subjectively assessed as reported by the patient and/or families in 3 domains, including emotional distress, limited normal feeding and restricted social activities, as the scores currently available for this assessment are not validated for Portuguese (9, 10). The criteria for defining EoE complications were adapted from Furuta *et al*. (18) and Gomez Torrijos *et al*. (19).

Laboratory and endoscopic analysis

Laboratory parameters (serum total IgE, peripheral blood eosinophil count) and findings in upper digestive endoscopy and the respective esophageal biopsies were also analyzed.

The diagnosis of EoE was performed using the established consensual clinical and histological criteria, which consider the presence of ≥ 15 eos/hpf in biopsy samples.

In this study, histological severe disease was defined by ≥ 55 eos/hpf and/or presence of microabscesses in esophageal biopsies. These criteria were adapted from van Rhijn *et al*., who divided patients in a group with high peak eosinophil count, characterized by 55-120 eos/hpf and a group with low peak eosinophil count characterized by 4-50 eos/hpf. This study showed that higher fibrotic and inflammatory signs scores in endoscopy, presence of esophageal stricture and eosinophilic microabscesses are associated with higher peak eosinophil counts (20).

Table I *- Demographic and clinical characterization of patients with eosinophilic esophagitis.*

Characteristics	EoE patients (n = 92)
Gender, n (%)	
Male	76 (83%)
Female	16 (17%)
Mean age, years [range]	
At the onset of symptoms	17 (SD 14) [1-63]
At diagnosis	21 (SD 15) [1-67]
Mean time gap between onset of symptoms and diagnosis	4 [0-3]
EoE diagnosis, n (%)	
< 18 years	45 (49%)
≥ 18 years	47 (51%)
Atopic comorbidities, n (%)	80 (87%)
Allergic rhinoconjunctivitis	76 (83%)
Food allergy	33 (36%)
Asthma	26 (28%)
Atopic dermatitis	12 (13%)

EoE: eosinophilic esophagitis; SD: standard deviation.

Statistical analysis

Statistical analysis was performed using Excel (Microsoft Office 2021) and IBM SPSS Software, Version 25.0 (IBM Corp.). Continuous variables were presented as means and standard deviations, or medians and interquartile ranges for variables with skewed distributions, and categorical variables as frequencies and percentages. Normal distribution was confirmed using Shapiro-Wilk test or skewness and kurtosis. For bivariate analysis, t-independent test or Mann Whitney test were used to compare parametric and non-parametric variables, respectively. Categorical variables were compared using Fisher's exact test or Pearson's chi-square (χ^2) test, as appropriate. P-values lower than 0.05 were considered statistically significant.

Results

Clinical characteristics

A total of 92 patients with eosinophilic esophagitis were included, of whom 76 were male (83%). Currently, twenty patients (22%) were < 18 years. Mean age at the onset of symptoms was 17 years (standard deviation (SD) 14, range 1-63) and at diagnosis 21 years (SD 15, range 1-67). It was observed a mean time gap between onset of symptoms and diagnosis of 4 years (range 0-31). In 47 (51%) patients' diagnosis was established at adult age and in 45 (49%) patients at pediatric age (< 18 years).

Most patients (n = 80, 87%) had a history of allergic disease: 83% (n = 76) allergic rhinoconjunctivitis, 36% (n = 33) food allergy, 28% (n = 26) asthma and 13% (n = 12) atopic dermatitis (**table I**).

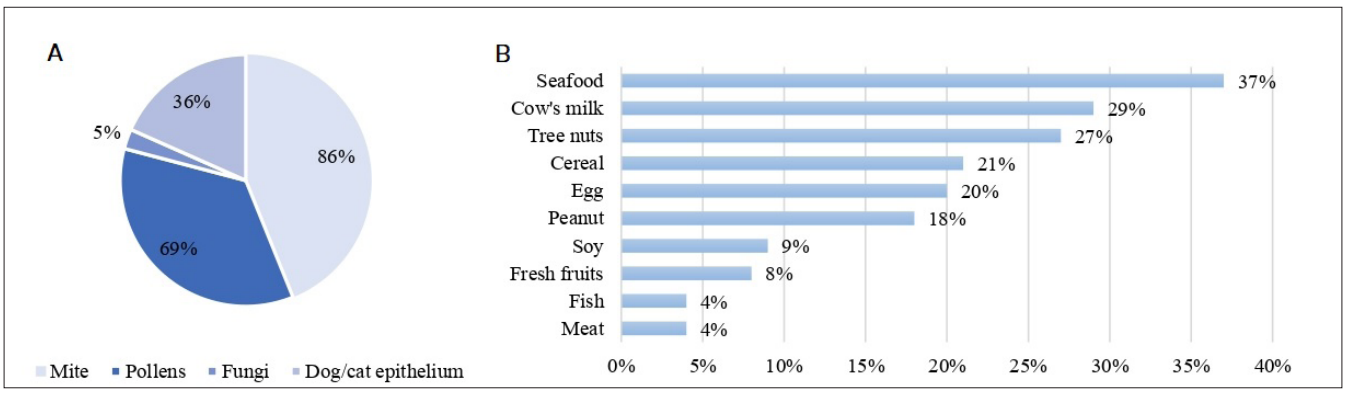
A seasonal spring exacerbation was considered when patients reported symptomatic esophageal complaints of EoE over this interval of time, translating flares of disease activity. Specifically, 6 (7%) patients allergic to tree and grass pollen reported clinical exacerbation in the spring months.

Patients with severe clinical disease had a significantly longer mean disease duration before diagnosis than patients without severe clinical disease (79 *versus* 15 months, respectively; p = 0.021). It was not demonstrated an association between disease duration before diagnosis and severe histological disease (p = 0.858).

Sensitization patterns

The skin prick tests with airborne allergens demonstrated a positive result in 77 (84%) patients. Among the patients with aeroallergens sensitization, house dust mites (n = 66, 86%) and pollens (n = 53, 69%) were the most common sensitivities observed (**figure 1A**).

Figure 1 *- Sensitization pattern of studied population.*



(A) Aeroallergen; (B) Food.

Sixty-five patients (71%) had food sensitization, demonstrated by skin prick tests in 45 patients (69%) and by food atopy patch tests in 34 (52%). All patients with food sensitization demonstrated by skin prick tests had also positive specific IgE. The foods with the highest frequency of sensitivity were seafood (n = 24, 37%), cow's milk (n = 19, 29%), nuts (n = 18, 27%) and cereals (n = 14, 21%). The complete distribution of food sensitization pattern of the studied population is represented in **figure 1B**.

Symptoms

The symptoms most frequently reported by patients and which led to the suspicion of the diagnosis of EoE were food impaction (n = 63, 68%), dysphagia (n = 56, 61%), gastroesophageal reflux (n = 40, 43%) and vomiting (n = 23, 25%), followed by chest pain (n = 19, 21%), abdominal pain/bloating (n = 17, 18%) and, at least, poor weight progression (n = 2, 2%). Both patients with impairment weight development were pediatric.

The inaugural symptom most reported was food impaction (n = 38, 41%), followed by dysphagia (n = 28, 30%), and vomiting (n = 13, 14%) (**figure 2**).

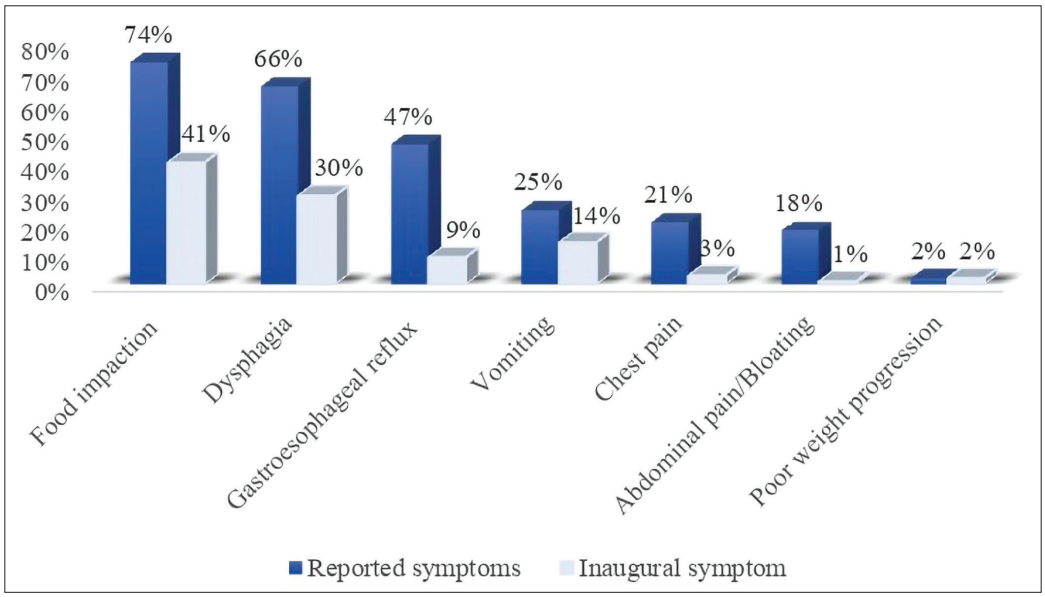
the most affected domain, and 49% (n = 25) had clinical complications requiring urgent medical care and/or hospitalizations. Patients who described food impaction (with or without the need for medical care) were significantly older at time of diagnosis than those who have never had impaction (median 18 years older *versus* 9 years older, respectively) ($p < 0.001$).

Laboratory parameters

The mean value of total IgE of the population was 525 IU/mL. When comparing the means of total IgE in the group of patients with food sensitization (629 IU/mL) and without food sensitization (188 IU/mL), there was a statistically significant difference ($p = 0.03$). Nevertheless, there was no statistically significant difference in the means of total IgE when comparing the groups with and without aeroallergen sensitization ($p = 0.194$).

The mean value of peripheral blood eosinophils was 489 cells/µL. There was no statistically significant difference in peripheral blood eosinophils mean values of patients with food sensitization and without food sensitization (537 cells/µL *versus* 502 cells/µL, respectively; $p = 0.29$). Similarly, there were also no

Figure 2 *- Description of symptoms reported by patients.*



In adult patients the most common presenting symptoms were food impaction (n = 28, 60%) and dysphagia (n = 15, 32%), whereas children presented predominantly with dysphagia (n = 13, 29%) and vomiting (n = 10, 22%).

Of the total of 51 patients with severe clinical disease (35 adults and 16 children), 88% (n = 45) reported a significant impact of the disease on quality of life, with emotional disturbance being

statistically significant differences in the mean peripheral blood eosinophil values between the groups with and without aeroallergen sensitization ($p = 0.408$).

No statistically significant difference was demonstrated between mean total IgE and mean eosinophil values and severe clinical or histological disease or presence of clinical complications ($p > 0.05$) (**table II**).

Table II *- Mean total IgE and mean eosinophil values according with severity.*

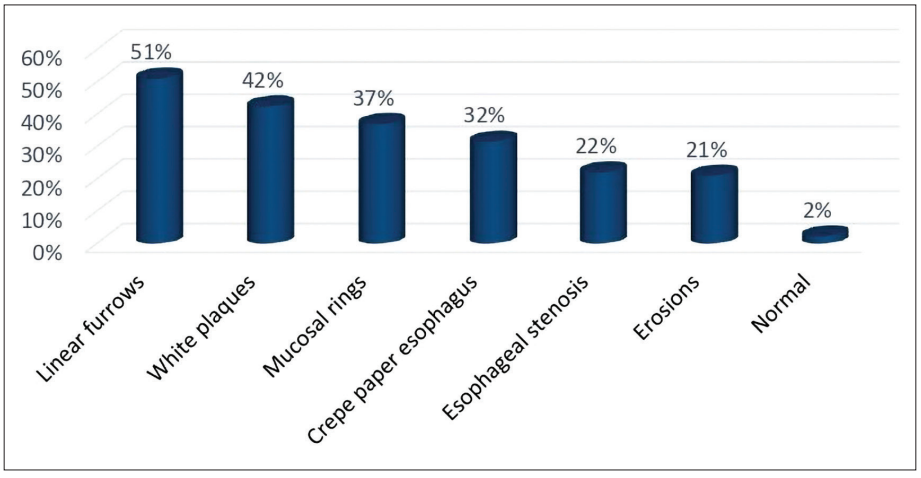
Mean value	Severe Clinic	No severe clinic	P-value	Clinical complications	No clinical complications	P-value	Severe histology	No severe histology	P-value
Total IgE (IU/mL)	398 (SD 123)	445 (SD 110)	0.653	415 (SD 120)	459 (SD 91)	0.771	334 (SD 85)	520 (SD 101)	0.07
Eosinophils (cells/µL)	457 (SD 73)	512 (SD 101)	0.349	431 (SD 51)	546 (SD 58)	0.182	436 (SD 39)	526 (SD 64)	0.718

SD: standard deviation.

Endoscopic and histological findings

In upper digestive endoscopy, linear furrows (n = 47, 51%) and white plaques (n = 39, 42%) were the most common detected findings. Esophageal stenosis was detected in 20 (22%) patients, and absence of alterations in 2 (2%) (**figure 3**).

Figure 3 *- Endoscopic findings in upper gastrointestinal endoscopy.*



When analyzing endoscopic findings by age, the most common were linear furrows (n = 25, 53%) and mucosal rings (n = 24, 51%) in adults and linear furrows (n = 22, 49%) and white plaques (n = 21, 47%) in pediatric age.

In the histological evaluation of biopsy samples, all patients had ≥15 eos/hpf, of whom 27 (29%) had ≥ 55 eos/hpf. Severe histological disease was identified in 34 (37%) patients, namely 21 (62%) children and 13 (38%) adults: ≥ 55 eos/hpf and/or presence of microabscesses. All histological features are represented in **table IIIA**. No association was found between ≥ 55 eos/hpf and/or presence of microabscesses on histology (severe histological disease) and clinical severe disease (p = 0.572).

Treatment and complications

In most patients (n = 68, 74%), combined treatment with swallowed fluticasone and high-dose proton pump inhibitor (PPI) was initiated. Twelve (13%) patients were medicated with isolated PPI and 11 (12%) with isolated swallowed fluticasone. Simultaneously, in 58 (63%) an elimination diet guided by positive food skin tests was instituted, and 2 (2%) patients were on an empiric elimination diet. One of the patients (1%) was not complying with any pharmacological treatment due to his own refusal, remaining asymptomatic on an elimination diet based on allergy skin tests but with no histological response (**table IIIB**).

Pre-diagnostic (n = 25, 27%) and post-diagnostic (n = 19, 21%) complications with urgent hospital admission were observed, in the latter mainly due to pharmacological treatment interruption (n = 14, 74%):

- Pre-diagnostic complications: 25 (27%)
- Post-diagnostic complications: 19 (21%)
  - Identified triggering factor: 15 (79%)
    - Pharmacological treatment interruption: 14 (74%)
    - Non-adherence to the elimination diet: 2 (11%)

Sensitization patterns and severity of disease

There was no significant association (p > 0.05) between aeroallergen or food sensitization and severe clinical disease, presence

***Table III - (A)** Characterization of histological evaluation of biopsy samples; **(B)** Characterization of performed treatment.*

(A)	
Histological evaluation	**n (%)**
15-54 eosinophils/hpf	65 (71%)
Basal zone hyperplasia	36 (39%)
Presence of microabscesses	28 (30%)
≥ 55 eosinophils/hpf	27 (29%)
Degranulation	21 (23%)
(B)	
Pharmacological treatment	**n (%)**
Swallowed fluticasone + PPI	68 (74%)
Isolated PPI	12 (13%)
Elimination diet	**n (%)**
Allergy testing-based*	58 (63%)
Swallowed fluticasone + PPI	51
Swallowed fluticasone	4
PPI	2
No pharmacological therapy	1
Empirical*	2 (2%)
Swallowed fluticasone + PPI	1
PPI	1

hpf: per high power field; *in association with pharmacological treatment; PPI: proton pump inhibitor.

of complications alone and severe histological disease. It was also not demonstrated an association between environmental aeroallergen (mite and pollens) and respective seasonal exacerbations.

Discussion and conclusions

This study included 92 patients diagnosed with EoE, both children and adults, of whom 83% were male (n = 76), which is in line with recent literature. Male predominance may be explained by a genetically male predisposition to develop this disease. There is a strong genetic component involved in the pathogenesis of EoE and a high concordance reported for EoE among family members. The pioneer study that described the genetic basis for EoE was a study of genome-wide microarray expression profile analysis, which reported that the gene responsible for EoE was TSLP (thymic stromal lymphopoietin) which is located in the 5q22 region of male X chromosome (21). EoE is generally diagnosed at least three years after the start of symptoms (22), consistent with our results. However, we have also noticed that some cases of EoE had a late diagnosis, remaining under-diagnosed for several years. This might explain the development of complications, such as food impactions, that represented the main symptom of the studied patients (n = 38, 41%), predominantly in adults (n = 32, 51%). Also, in our population it was demonstrated that patients with severe clinical disease had a significantly longer disease duration before diagnosis than patients without severe clinical disease and that patients who described food impaction (with or without the need for observation in Emergency Department and/or hospitalization) were significantly older at time of diagnosis than those who have never had impaction.

This delay in diagnosis emphasizes the importance of actively searching for patients with compatible symptoms, as an early diagnosis is crucial to avoid the development and progression of disease. Noteworthy, according to literature, the pathogenic process that leads to esophageal remodeling, may goes on to develop clinically relevant long and short segment narrowing, with progression of disease, as in our patients, but some other times may lead to direct mucosal healing without sequelae. In favor of this, CAPN14 has been implicated in eliciting and repairing the epithelial damage associated with EoE, suggesting that remodeling could involve a genetically controlled balance between these two processes (21).

In view of the demonstrated ability of aeroallergens to trigger and/or exacerbate EoE and strong association of EoE with atopy it is recommended assessment of sensitization profile in all patients (5). There is recent evidence that suggests that EoE may be an element of the allergic march that classically begins with atopic dermatitis and progresses to IgE-mediated food allergy, asthma, and allergic rhinitis (6). Most patients have a history of atopy, having been observed a high prevalence in the studied population (n = 80, 87%). Allergic respiratory disease (allergic rhinoconjunctivitis and asthma) affected 83% (n = 76) and 28% (n = 26) of our patients, respectively. The values described in the literature are similar to those presented (3).

Seventy-seven (84%) patients in this population presented aeroallergen sensitization, more frequent to house dust mites (n = 66, 86%) and pollens (n = 53, 69%).

When analyzing data on food sensitization, it is known that IgE-based testing does not reflect the triggering mechanism in patients with EoE and atopy patch testing, although reflecting the delayed-type hypersensitivity mechanism of EoE is not standardized or validated. Despite there was considerable heterogeneity between studies, attributable to varying methods of allergy testing, a meta-analysis that combined the results of all studies using allergy-based testing to identify food triggers in patients with EoE provided a summary estimate for effectiveness in achieving histological remission of 32.2% in adults and 47.9% in children (23, 24). Also, according to a systematic review of 16 studies published between 2002 and 2020, the positive predictive value (PPV) of the SPT+APT combination was 67.1%, with 65-88.3% of patients presenting symptom improvement

after following a SPT+APT-based elimination diet (25). The combination of SPT+APT+sIgE was studied only by Dalby *et al*. (26) stating symptoms' improvement in 67% of patients. It can be presumed that the detection of both humoral and cellular sensitization to food allergens and the food elimination of all allergens with positive results from any of the allergy methods offers an increased PPV. Considering these results and once difficulties in patients' compliance with empirical avoidance diets are real and frequently observed in our clinical practice, we think that allergy tests, SPT and APT, may continue to be considered, especially in children, as a complement to first-line pharmacological therapy (swallowed corticosteroids and/or PPI) and not as a replacement for it (10, 24). Additionally, SPT are particularly important to confirm or exclude the suspected diagnosis of concomitant IgE-mediated food allergy (25).

Sixty-five (71%) patients presented food sensitization, and we observed being the most prevalent sensitization to seafood (n = 24, 37%), cow's milk (n = 19, 29%), nuts (n = 18, 27%) and cereals (n = 14, 21%). These are atypical sensitizations, as we would expect the most prevalent sensitizations to be to milk, egg, wheat, soy, and peanuts, which according to European EoE patients' series are the most frequently reported as food allergies (5, 8).

As widely described, presenting symptoms vary according to age, as previously mentioned. In younger children, nonspecific symptoms, including eating refusal, nausea and vomiting are more frequent, whereas in older children, teenagers and adults, the predominant symptoms are mechanical complaints, such as dysphagia and food impaction (27). We have observed in pediatric age a predominance of dysphagia (n = 13, 29%) and vomiting (n = 10, 22%), and in adults food impact (n = 32, 51%) and dysphagia (n = 19, 30%). As pediatric age includes both younger children and teenagers, these findings are in conformity with the published data. Reported symptoms also included non-specific suggestive complaints of gastroesophageal reflux (n = 40, 43%) and episodes of chest pain (n = 19, 21%).

Most EoE patients have elevated serum total IgE levels (for a reference value < 100 KUA/L) and about 50% have peripheral eosinophilia (for a reference value < 300 cells/μL) (1, 3, 21). The mean value of total IgE of the population was 525 IU/mL, having been evidenced a statistically significant higher value ($p = 0.03$) in the group of patients with food sensitization (629 IU/mL), when compared to without food sensitization (188 IU/mL). As well as the mean total IgE value, the mean value of eosinophils was also elevated (489 cells/μL). No difference was demonstrated between these values and disease severity ($p > 0.05$). The physical examination of patients with EoE is often completely normal, so the diagnosis of EoE is dependent not only on the clinical manifestations and high degree of suspicion, but also on the endoscopic findings and histological examination of esophageal mucosal biopsies (27). Endoscopic features can be subtle and missed on endoscopy, and therefore multiple esophageal biopsies, are required in all patients suspected of having EoE, irrespective of endoscopic appearance, with recommendations ranging between four and six, in which six biopsies correspond to a 97%-100% chance of making a positive diagnosis. Therefore, latest guidelines recommend that six biopsies should be taken from at least two different sites in the esophagus and that these six biopsies should include a combination of targeted biopsies from visible areas of mucosal surface abnormality, whenever they are apparent, and non-targeted biopsies (24, 28, 29). The most common endoscopic features in adults with EoE include linear furrows and mucosal rings. Children often present with similar endoscopic features, but the findings may be more subtle. In a large clinical series of 381 children, the most common endoscopic features were normal appearance (32%), linear furrows (41%), esophageal rings (12%), and white plaques (15%) (30). When evaluating this population endoscopic findings by age, the most common were linear furrows (n = 25, 53%) and mucosal rings (n = 24, 51%) in adults, which is in accordance with the data in literature. In pediatric age linear furrows (n = 22, 49%) and white plaques (n = 21, 47%) were the most frequent. Only 2% (n = 2) had a normal result in endoscopic examination, a lower percentage than the described, which might indicate a delay in referral and diagnosis of these children.

Histology is similar between children and adults, although collagen deposits increase with the patient's age. Biopsies are currently mandatory to evaluate eosinophil infiltrates and optional additional histological markers such as eosinophils microabscesses or basal zone hyperplasia. In addition, biopsies from the duodenum and stomach should be collected to rule out eosinophilic gastroenteritis (29). It must be highlighted that severe histological disease was identified in 34 (37%) patients, defined by ≥ 55 eos/hpf (n = 27, 29%) and/or presence of microabscesses (n = 28, 30%). The presence of ≥ 55 eos/hpf on histology was not associated with a more severe clinical disease.

The practical goals of treatment of EoE are to prevent complications, stabilize the disease, and
reverse fibrosis. Whether this last goal is achievable by current treatment is unclear (9).

Pharmacologic therapy consists of either PPIs and/or topical steroids. High doses of PPIs given twice daily for 8 weeks are 40% to 60% effective for achieving histologic remission. The mechanism of PPI efficacy seems to be at least partly independent of acid suppression and perhaps due to anti-inflammatory effects and restoration of esophageal barrier function. Topical steroids have efficacy of 60% to 95% (30, 31). Dupilumab is the only medical therapy to date that has been approved by the U.S. Food and Drug Administration to treat eosinophilic esophagitis. It has recently been approved in patients 12 years of age and older weighing at least 40 kilograms (32, 33).

In agreement with published recommendations, the majority of patients (n = 68, 74%) were medicated with a combined treatment with swallowed fluticasone and proton pump inhibitor (PPI), 12 (13%) with isolated PPI and 11 (12%) with isolated swallowed fluticasone.

Regarding efficacy of food elimination diets (FED), another available therapeutic option, results in literature are heterogeneous according to different studies, even referring to the same diet.

A meta-analysis by Arias *et al*. reports that the effectiveness of an amino-acid-based elemental diet is approximately 90% in both children and adults, and the 6-FED show effectiveness for 72.1% of cases, while the allergy-test-directed elimination has shown efficacy in only 45.5% of the cases. However, in a recently published systematic review by Dalby *et al*., formerly stated, allergy-test-driven elimination diets are effective in 66-88.3% of the cases, similarly to empirical diets (25, 34).

As efficacy of allergy-test-driven elimination diets do not seem to be superior to empiric FED based on most involved foods or food groups (*e.g*., two-, four-, and six-food diets), we assume that the decision any of these options, or alternatively a 4- or 2-FED (corresponding respectively to 54%-64% and 43% response rates), are valid and should be individualized and selected according to patient's diet adherence and lifestyle (24, 25).

Elemental diets produce histologic remission in most patients. However, practical limitations limit their use (*e.g*., cost, palatability, gastrostomy tube placement, quality of life). From our experience, allergy test-directed diets have a significantly higher rate of adherence in clinical practice. It should be noted that a higher percentage of our patients were on allergy test-directed diets (n = 58, 63%) compared to empiric elimination diets (n = 2, 2%). This low number reflects the challenges in long-term adherence to 6-food elimination diet (34, 35). Both patients started this diet as indicated by Gastroenterology. One of them was referred to Immunoalergology for study due to lack of clinical improvement and histological remission despite treatment with swallowed corticosteroids and PPI, admitting not being able to carry out the suggested dietary restrictions. The patient had a history suggestive of cow's milk IgE-mediated allergy and SPT were positive to cow's milk, egg, fish and shellfish, and negative for wheat, soy, tree nuts and peanut. The patch tests were positive for shrimp. He started an avoidance diet based on these results, which he managed to comply, and the intake of wheat, soy, nuts and peanuts was liberalized. A symptom resolution has been reported, and a histological remission was observed. The other patient presented histological remission under therapy with swallowed corticosteroids, PPI and 6-FED but, due to the difficulty of maintaining adherence to the established diet, with a significant negative impact on quality of life, he was referred to Immunoallergology to consider less restrictive diet based on allergy skin tests. There was no history of IgE-mediated food allergy and allergy tests were negative to the standard panel of food allergens tested, namely, milk and proteins (alfa-lactoalbumin, beta-lactoglobulin, casein), soy, egg (yolk, white, ovalbumin, ovomucoid), meats (beef, chicken, turkey, pork and rabbit), fish (bream, hake, axillary seabream, sea bass, sardine, horse mackerel, cod, tuna, salmon), seafood (shrimp), cereals (rice, wheat, barley, corn, rye, and oat), fresh fruits (apple, pear, and peach), tree nuts (walnut, hazelnut, cashew, almond, chestnut, pistachio, pinion) and peanut. It was decided to start a 4-food empiric elimination diet, allowing the intake of fish, shellfish, nuts and peanuts, which proved to be effective in recent studies, providing 54-64% of effectiveness in terms of inducing clinical and histological remission of EoE (23). This patient reported quality of life improvement, managed to maintain the diet and maintained remission of the disease.

In order to tailor an individual and specific treatment, we assumed it would be important to know whether sensitization to airborne or food allergens is relevant for causing and/or eliciting a more severe disease. According to the latest adult and pediatric guidelines of the British Society of Gastroenterology and British Society of Paediatric Gastroenterology, Hepatology and Nutrition there are links to EoE exacerbations during pollen season, associated with higher pollen exposure (low grade evidence). However, definite conclusions on the causal association of seasonality and aeroallergen exposure remain to be established (24). In our study, there was no demonstrated association between airborne sensitization patterns and clinical and histological severity, nor between food sensitization patterns and disease severity, which has also been published in the scarce available literature (36, 37).

To conclude, in our population of patients with EoE, like others, it was observed a predominance of male patients, and a high prevalence of atopy, with a high sensitization to environmental aeroallergens and food allergens. Severe situations of food impaction were more frequent as a presenting symptom in adults, presumably because of esophageal remodeling that occurs with the progressive evolution of the disease, which may develop clinically relevant long and short segment narrowing. Children presented predominantly with dysphagia.

At the time of diagnosis, approximately half of the patients presented severe clinical disease (55%), of which 88% reported a significant disease burden on quality of life, and one third severe histological disease (37%). According to our results, an older age at diagnosis and a longer disease duration before diagnosis appear to be useful for predicting EoE clinical severity, whereas specific sensitization patterns, serum total IgE and peripheral blood eosinophil values do not. No association was found between histological and clinical severe disease. Our study draws attention to the need for education/training on EoE, to optimize early diagnosis and treatment, reduce associated real comorbidities and/or complications, and increase patient's quality of life.

More studies with large numbers of patients are needed to better understand this disease and its mechanisms.

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Contributions

LEC, RL, CC: conceptualization. LEC, RL, RB: investigation. LEC, R.L: formal analysis, writing - original draft. LEC, RL, RB, EP, CC: writing - review & editing. CC: resources.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Homa Sadri[1], Atossa Madanipour[2], Negar Toami[3], Mahmood Bakhtiari[1], Hadi Montazerlotfelahi[4], Ehsan Zahmatkesh[3], Atefeh Zandifar[5], Marzieh Tavakol[1,*]

A survey on the association of seizure disorders with asthma and allergies in children

[1]Non-Communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran
[2]Student Research Committee, Alborz University of Medical Sciences, Karaj, Iran
[3]Department of Pediatrics, Imam Ali Hospital, Alborz University of Medical Sciences, Karaj, Iran
[4]Department of Pediatric Neurology, Imam Ali Hospital, Alborz University of Medical Sciences, Karaj, Iran
[5]Department of Psychiatry, Imam Hossein Hospital, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
[*]Second corresponding author: marziyeh.tavakol@gmail.com

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Corresponding author
Homa Sadri
Non-Communicable Diseases Research Center
Alborz University of Medical Sciences
Karaj, Iran
ORCID: 0000-0002-1451-0410
E-mail: homasadri7@gmail.com

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Impact statement

Little is known about the relationship between allergic diseases and seizure disorders. The number of comorbid allergic diseases is more related to the development of types of seizures than types of allergic disease.

Summary

Background. *Little is known about the relationship between allergic diseases and seizure disorders including epilepsy. It is hypothesized that inflammation from allergic diseases may predispose children to seizures. The aim of this study is to investigate the frequency of seizure disorder in children with asthma and allergy.* ***Methods.*** *This is a cross-sectional survey study of parents of 1,300 children and adolescents under 20 years of age referred to the Allergy and Asthma Clinic of Imam Ali Hospital (Karaj) who were asked to complete a screening questionnaire for seizures in their children. Parents who reported any history of seizures in their children were contacted to answer a second in-depth questionnaire to determine more detail of type, triggers, and treatment of seizures.* ***Results.*** *A total of 705 males (62%) and 433 females (38%) participated in this study, with a mean and standard age of 6.62 ± 4.57 years. Among them, 70.6% had asthma, 15.2% had allergic rhinitis, 5.6% had atopic dermatitis, 3.5% had urticaria, 2.7% had food allergies, 1% had drug allergies, and 1.4% had other allergic diseases. Additionally, 88 patients (7.7%) had a history of doctor-diagnosed seizures, 57 patients (5%) had febrile convulsions, and 15 patients (1.31%) had idiopathic epilepsy. There was no significant relationship found between febrile convulsions, seizures, and epilepsy with the type of allergic diseases. However, a significant association was observed between the number of comorbid allergic diseases in patients with febrile convulsions (OR 1.4, 95%CI 1.07-1.83, p = 0.013). There was also an association between the epilepsy and comorbid allergic diseases number with an OR 1.84, 95%CI 0.28-12, however the risk of epilepsy was increased by 0.84% but this increase was not significant. Regarding the relation between the number of allergic diseases in parents and idiopathic epilepsy in their children, a significant association was found only for mothers (OR 1.28, 95%CI 1.04-2.23, p = 0.024), but not for fathers (p > 0.05).* ***Conclusions.*** *Febrile convulsion is associated with the number of comorbid allergic diseases in children and the mother's number of allergic diseases is more related to idiopathic epilepsy in children than the father's.*

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Introduction

Allergic diseases including asthma, allergic rhinitis, atopic dermatitis and food allergy are the most common chronic health conditions in the world. These diseases affect approximately 20% of the global population worldwide now (1), only respiratory allergic diseases, namely asthma and allergic rhinitis, affect approximately seven hundred million people (2).

Allergic diseases such as atopic dermatitis (AD) and asthma are associated with multiple neuropsychiatric and mental health disorders in children and adults, including depression, anxiety, attention deficit hyperactivity disorder and autism (3-6). Despite growing evidence of such a connection, little is known about the relationship between allergic diseases and other neurological disorders, including epilepsy. Seizures are one of the most common neurological disorders in children. In Iran, the lifetime prevalence of childhood seizure has been reported to be 3.2%, and approximately 60% of these cases were related to convulsions caused by fever (7).

Many adults with epilepsy experience the onset of seizures in childhood. Almost a quarter of children with epilepsy are drug-resistant, and the exact pathophysiology of epilepsy is still unknown, but research has determined the importance of neuroinflammation and related cytokines in this regard (8, 9). A meta-analysis revealed a positive link between several different epilepsy etiologies and elevated levels for IL-1ra, IL-1β, IL-6 and CXCL8/IL-8. It is also reported the increased levels of IL-1α, IL-7, and IL-13, as well as the chemokines CCL2-5, -19, and -22, exclusively in brain tissue (9). Other studies have also shown the importance of the relationship between microglial cells and mast cells in nervous system inflammation (5).

Animal studies show that the inflammatory pathways involved in allergic diseases may also be activated in the brain and contribute to the development of epilepsy (10). Previous epidemiological studies have shown an association between epilepsy and asthma in adults (11, 12), but the mechanism of association between allergic disease and seizures is still unclear. Perhaps inflammation caused by allergic disease exposes children to seizures. Overall, there are very few studies on the relationship between epilepsy and allergic diseases, especially in childhood, and research on the relationship between these two diseases is new. The aim of this study is to investigate the frequency of seizure disorder in children with asthma and allergy.

Materials and methods

This study was done in a cross-sectional manner. The method in this study was availability sampling. The patients were selected from allergy clinics of Imam Ali Hospital in Karaj. The code of ethics (IR.ABZUMS.REC.1398.138) was obtained from the Ethics Committee of Alborz University of Medical Sciences.

In this study, 1,300 patients with asthma and other allergic diseases under 20 years of age who referred to the allergy clinics of this center in 2017-2018 and met the inclusion criteria and did not have the exclusion criteria were selected. Inclusion criteria included age below 20 years, parental and patient consent to participate in the study, and diagnosis of allergic disease/asthma by an asthma and allergy specialist. Exclusion criteria included lack of consent of the patient or their parents to participate in the study and the reason for referral was other than allergic diseases. After obtaining the consent form from these 1,300 patients and explaining the plan by the researcher, the questionnaire was completed for the patients. In this questionnaire, you will be asked about demographic information including age, gender, and place of residence. Then, you will be asked about the reason for the referral and the type of allergic disease or diseases, including asthma, nasal allergy, eye allergy, eczema, urticaria, food allergy, drug allergy, and pruritus. The diagnosis of allergic disease or asthma is confirmed by an asthma and allergy specialist. Then, the patients or their parents were asked about the "history of mentioned allergic problems in first-degree relatives" and "history of seizure diagnosis by doctor in the child". If the patient had a positive history of seizures, a second questionnaire was given to the patients, asking questions such as the age of the first onset of seizures, recurrence of seizures, accompanying seizures with or without fever, states during seizures, cause of them, history of drug use, drugs currently used, the history of drug sensitivity following the use of anticonvulsant drugs, other neurological diseases and the developmental status.

In this study, if a patient with normal neurologic physical exam and brain MRI had more than one seizure without an underlying cause such as fever, metabolic or infectious diseases, it was classified as idiopathic epilepsy, and if there was an identifiable cause for repeated and recurring seizures, it was classified as chronic symptomatic epilepsy. If the onset of convulsions in a child was accompanied by fever and in the age range of 6 months to 6 years without central nervous system infection or other predisposing disorders, it would be classified febrile convulsions. After completing the questionnaires, the type of seizures in children was categorized based on the information recorded by the parents, the consultation, and opinion of a pediatric neurology specialist. At the end, the data were collected and subjected to statistical analysis, and the obtained statistics were finally compared with the global average statistics of childhood seizures.

Statistical analysis

Continuous baseline demographic and clinical data are presented as mean ± standard deviation and grouped data as frequencies and percentages. The comparison of means between the groups, for quantitative variables with approximately normal distribution, was performed using paired or independent t-tests

and one way ANOVA as appropriate. Chi-square test or Fisher's exact test were used to determine the independence of the two categorical variables. P-value less than 0.05 was considered as significant. All analysis ere done using SPSS software version 24.

Results

Out of 1,300 patients, 45 patients were excluded due to lack of proper cooperation and 52 patients due to repetition, 35 patients due to incomplete and unreliable information, 10 patients due to declaration of lack of consent to participate in the study, and 20 patients due to immunodeficiency; after completing the questionnaires they were excluded from the study, and we obtained the results from the information of 1,138 patients. The average age of the patients was 6.62 with a standard deviation of 4.57, and the minimum and maximum age was 0.5 and 20 years (**table I**).

In terms of gender, 705 patients (62%) were boys, and 433 patients (38%) were girls, which were significantly different in Fisher's exact test ($p < 0.05$).

Regarding the reason for visiting the clinic, 803 patients (70.6%) had asthma/airway hyperreactivity, 173 patients (15.2%) had allergic rhinitis, 64 patients (5.6%) had eczema, 40 patients (3.5%) with urticaria, 31 patients (2.7%) with food allergy, 11 patients (1%) with drug allergy, 8 patients (0.7%) with chronic itching, 7 patients (0.6%) with ocular allergic disease and 1 (0.1%) patient had anaphylactic reaction. 719 patients (63.2%) had one allergic disease, 272 patients (24.1%) had two allergic diseases, 85 patients (7.5%) had three allergic diseases, and 60 patients (5.3%) had 4 allergic diseases.

In terms of the history of atopy in the parents, in 596 patients (52.4%) no history of allergic disease was reported in any of the parents, in 542 patients (47.6%) the history of atopy was seen in the parents of the patients.

The frequency of seizure history in patients according to the history of atopy in parents had no statistically significant difference in Fisher's exact test ($p > 0.05$).

Regarding the history of seizures in the patient's relatives, 166 people (14.6%) had a positive history and 972 people (85.4%) had a negative history.

Out of 166 people with a history of positive seizures in the patient's relatives, 86 people (7.6%) had first degree, 55 people (4.8%) had second degree and 25 people (2.2%) had third degree.

In terms of history of seizures in patients, 88 patients (7.7%) had a positive history and 1,050 (92.3%) had a negative history of seizures, and the frequency of history of seizures in patients according to the history of seizures in relatives according to Fisher's test had a significant difference ($p < 0.05$).

Regarding the number of seizures, 58 patients (65.9%) had only one seizure and 30 patients (34.1%) had more than one seizure.

Regarding the occurrence of seizures in the last 5 years, 57 patients (64.8%) had seizures in the last 5 years and 31 patients (35.2%) did not have seizures in the last 5 years.

Regarding anticonvulsant drug use in patients with a positive history of seizures, 64 patients (72.7%) were not currently taking any anticonvulsant drug, 13 patients (14.8%) were taking an anticonvulsant drug, 9 patients (10.2%) were taking at least two anticonvulsant drugs, and their seizures were controlled, and 2 patients (2.3%) were taking at least two anticonvulsant drugs and their seizures were not controlled.

In terms of accompanying fever with seizures in patients with a history of seizures, in 57 patients (64.8%) seizures were always accompanied by fever, in 27 patients (30.7%) seizures were without fever and in 4 patients (4.5%) seizures were sometimes with fever.

In patients with a positive history of seizures, in 57 patients (64.8%) seizures accompanied by fever, in 3 patients (3.4%) with head trauma, in 2 patients (2.3%) with meningitis/encephalitis, in 1 patient (1.1%) with metabolic diseases, in 1 patient (1.1%) with drug poisoning, in 1 patient (1.1%) with brain tumor, in 1 patient (1.1%) with vaccine, in 1 patient (1.1%) with neonatal seizures and 21 patients (23.9%) had epilepsy. Of the 21 patients with epilepsy, 15 patients (1.31%) had

Table I *- The frequency of patients with seizure history according to age group.*

	Positive history number (percentage)	Negative history number (percentage)	P-value
Age			0.246
Less than 1 year	6 (4.3%)	134 (95.7%)	
Between 1 and 2 years	5 (4.7%)	101 (95.3%)	
2 to 5 years	19 (7.4%)	237 (92.6%)	
6 to 12 years	46 (9.1%)	458 (90.9%)	
More than 12 years	12 (9.1%)	120 (90.9%)	

Table II *- Frequency of patients in terms of history of seizures according to gender.*

	Positive history number (percentage)	Negative history number (percentage)	P-value
Gender			0.005
Boys	66 (9.4%)	639 (90.6%)	
Girls	22 (5.1%)	411 (94.9%)	

idiopathic epilepsy (occurrence of seizures more than once and without any underlying factors) and the other 6 patients were classified as chronic symptomatic epilepsy.

Therefore, in general, the frequency of history of seizures with fever in the population of allergic children referring to Imam Ali Clinic was 50 per 1,000 people, and the frequency of idiopathic epilepsy in this population was 13 people per 1,000 people.

Regarding the change of anticonvulsant medication in patients with a history of seizures, 23 patients (95.8%) did not change or stop taking the medication due to an allergic reaction, and 1 patient (4.2%) stopped taking the anticonvulsant medication due to an allergic reaction without requiring hospitalization.

The frequency of history of seizures in patients according to the gender of the patients is shown in **table II**, which had a significant difference in Fisher's exact test ($p < 0.05$).

The frequency of seizure history in patients according to the allergic disease of the patients is shown in **table III**, which had no significant difference with each other ($p > 0.05$).

The frequency of seizure history in patients was checked according to the number of allergic diseases of the patients, which did not have significant differences with each other ($p > 0.05$).

Also, the frequency of idiopathic epilepsy in the patients according to the type of allergic disease was not significantly different from each other ($p > 0.05$).

There was an association between the epilepsy and allergic disease number with an odds ratio OR 1.84, 95%CI 0.28-12, however the risk of epilepsy was increased by 0.84%, but this increase was not significant.

The frequency of idiopathic epilepsy in patients according to the number of comorbid allergic diseases of the patients' fathers had no statistically significant difference ($p > 0.05$).

Table III *- The frequency of patients in terms of seizure history according to the allergic disease of the patients.*

	Positive history of seizure number (percentage)	Negative history of seizure number (percentage)	P-value
Disease			0.602
Asthma/Airway hyperreactivity	64 (8%)	739 (92%)	
Allergic rhinitis	15 (8.7%)	158 (91.3%)	
Eye allergy	0 (0%)	7 (100%)	
eczema	1 (1.6%)	63 (98.4%)	
Urticaria	5 (12.5%)	35 (87.5%)	
Chronic itching	0 (0%)	8 (100%)	
Food allergy	2 (6.5%)	29 (93.5%)	
Drug allergy	1 (9.1%)	10 (90.9%)	
Anaphylaxis	1 (100%)	0 (0%)	

Table IV *- The frequency of patients in terms of idiopathic epilepsy according to the number of comorbid allergic diseases.*

	Positive Idiopathic epilepsy number (percentage)	Negative Idiopathic epilepsy number (percentage)	Total
Number of allergic diseases in patients			
One	13 (1.81%)	706 (98.19%)	719
Two	0 0%)	274 (100%)	274
Three	0 (0%)	85 (100%)	85
Four	2 (3.33%)	58 (96.67%)	60

Table V - *The frequency of idiopathic epilepsy in patients according to the number of allergic diseases of the patients' parents.*

	Number of allergic diseases in mothers		Number of allergic diseases in fathers	
	Positive idiopathic epilepsy number (percentage)	**Negative idiopathic epilepsy number (percentage)**	**Positive idiopathic epilepsy number (percentage)**	**Negative idiopathic epilepsy number (percentage)**
No allergy	12 (1.49%)	791 (98.51%)	13 (1.55%)	827 (98.45%)
One allergic disease	1 (0.36%)	273 (99.64%)	2 (0.77%)	258 (99.23%)
Two allergic diseases	0 (0%)	32 (100%)	0 (0%)	23 (100%)
Three allergic diseases	1 (5%)	19 (95%)	0 (0%)	10 (100%)
Four allergic diseases	1 (11.11%)	8 (88.89%)	0 (0%)	5 (100%)
P-value	0.024		> 0.05	

But the frequency of idiopathic epilepsy in patients according to the number of allergic diseases of the patients' mothers had a statistically significant difference in the Chi-square test (p = 0.024, OR 1.28, 95%CI 1.04-2.23) (**table IV**).

Next, the frequency of febrile convulsion in patients according to the allergic disease of the patients is shown in **table V**, which were not significantly different from each other in the chi-square test (p > 0.05).

Next, the frequency of febrile convulsion in patients according to the number of allergic diseases of patients is shown in **table VI**, which had a significant association between the number of comorbid allergic diseases in patients with febrile convulsions (OR 1.4, 95%CI 1.07-1.83, p = 0.013).

The frequency of febrile convulsions in children had no significant relationship with the number of allergic diseases of neither father nor mother.

Discussion and conclusions

In this study, in terms of history of seizures in patients, 88 patients (7.7%) had a positive history, and 1,050 patients (92.3%) didn't have, and regarding the subgroups of seizures, 21 patients had epilepsy (15 of them had idiopathic epilepsy) and 57 patients had febrile convulsion. Therefore, the prevalence of epilepsy, idiopathic epilepsy, and febrile seizures were 18.4/1,000, 13/1,000, and 50/1,000, respectively.

In Iran, the lifetime prevalence of epilepsy has been reported 16.6 per 1,000 people with an average age of onset of 21.1 ± 19.1 years, and 50.6% of patients had an underlying cause (13). Although this study was not limited to the age group of children, the prevalence of idiopathic epilepsy was lower than in the present study.

Some studies have been conducted on the prevalence of epilepsy in the population of children in our region. In a study from Bahrami Hospital in Tehran (7), which was reported in 2009,

Table VI - *The frequency of patients in terms of febrile convulsion according to the number of comorbid allergic diseases.*

	Positive history of febrile convulsion number (percentage)	**Negative history of febrile convulsion number (percentage)**	**Total**	**P-value**
Number of allergic diseases in patients				0.013
One	20 (2.78%)	699 (97.22%)	719	
Two	30 (10.95%)	244(89.05%)	274	
Three	4 (4.71%)	81 (95.29%)	85	
Four	3 (5%)	57 (95%)	60	

the history of seizures in 2,500 children under the age of 12 was questioned, and the prevalence of history of seizures in this population was 32/1,000, the prevalence of history of epilepsy was 4.4 in 1,000, and the prevalence of febrile convulsion was 19/1,000, which is significantly lower than the present study. Part of the difference between the results of this study and ours is due to the target population of the study. In Alizadeh *et al.*'s study (7), the prevalence of seizures in school children was evaluated, and thus, a part of the population of children who are unable to go to school, such as cerebral palsy patients (who are at a higher risk for seizures) were excluded from the study. But this problem does not exist in the case of children referred to the allergy clinic. Considering the very small number of these patients in the allergy clinic, it seems that the reason for this significant difference in the prevalence of seizures between the two studies is beyond this issue.

In another study from Turkey, the prevalence of epilepsy in 4,288 children aged 0-17 years was investigated, and the prevalence of epilepsy in this population was reported as 8.6 per 1,000 people (14). Another study from Egypt, among 36,195 children and adolescents under 18 years of age, the overall prevalence of epilepsy was 9.7/1,000, but the prevalence of idiopathic epilepsy in this population was 5.7/1,000 (15). Therefore, compared to regional studies on the general population of children, it seems that the history of seizures and idiopathic epilepsy are more frequent in allergic children. However, it would be better to evaluate the frequency of history of seizures and epilepsy in the general population of children and adolescents under 20 years of age in Karaj to make more reliable comparisons and conclusions.

On the other hand, the frequency of history of epilepsy in patients with at least one allergic disease in 2014 in America out of 91,605 patients was reported as 1.37%, which is equivalent to 13.7/1,000 (16), and in our study, the prevalence of idiopathic epilepsy was about 13 per 1,000 people, which is very close to this study. In Strom *et al.*'s study (17), according to their evidence, they have come to the conclusion that there is a positive association between allergic disease and seizures, which the present study could not show and the reason for this difference can be due to the difference in the sample size, the geographical area and the difference in some definitions in the two studies.

In this study, the frequency of males in patients with a history of seizures was significantly higher than that of females, and the results of Silverberg *et al.*'s study (16) show the same relationship. On the other hand, in the Strom *et al.*'s study (17), the most frequent patients with a history of seizures were seen in patients with food allergies, while in the present study, it was seen in patients with urticaria (not statistically significant). Of course, it should be noted that in children urticaria can be related to food allergies, and therefore, from this aspect, patients need more careful follow-up to identify the cause of urticaria. Also, in the present study 5.6% of the patients had eczema, and among the 64 patients with eczema, one patient had history of seizures, while in Strom *et al.*'s study, 9.5% of the patients had eczema and among 19,647 patients with eczema, 263 patients had a history of seizures (prevalence 1.4%) (17). In Silverberg's study (16), the prevalence of epilepsy in children with eczema was 1.73%. Also, in the Taiwan study, the prevalence of epilepsy in atopic dermatitis patients compared to the control group was 0.94 to 0.27 cases per 1,000 people per year (18). The estimation of seizure prevalence in the atopic dermatitis population participating in our study has low statistical power due to the limitation of the sample size. In addition, since many children with asthma and allergic rhinitis also have a past history of atopic dermatitis and food allergy, and in this study only the current allergic diseases of the patients were recorded, maybe if the past history of atopic diseases was also taken into account, the results would be different.

In terms of the prevalence of epilepsy in the population with asthma, in the current study, out of 803 patients with asthma, 9 cases have idiopathic epilepsy, which is a prevalence of 11.2/1,000, which is higher than the statistics calculated in Castaned's study (19). A recent cohort study including 150,827 patients with asthma showed that the probability of developing epilepsy in patients with asthma was 1.39 times higher than in healthy controls (11).

In a Mendelian randomization (MR) study whether asthma causally contributes to epilepsy susceptibility was investigated and they reported asthma is associated with an increased risk of epilepsy independent of the age onset of asthma (20). The potential reasons for the association between asthma and epilepsy could be due to anoxia and hypocapnia caused by frequent asthma attacks, or perhaps chronic inflammation is a common pathological feature of asthma and epilepsy.

Silverberg *et al.* (16) reported that as the number of comorbid allergic diseases increased, so did the odds of both lifetime and point prevalence of epilepsy. It was reported by Strom *et al.* (17) that childhood seizures were associated with 1, 2, and 3 comorbid allergic diseases but were most strongly associated with 4 comorbid allergic diseases.

In the current study, there was an association between the epilepsy and number of comorbid allergic diseases, with an odds ratio of 1.84, 95%CI 0.28-12. The frequency of idiopathic epilepsy was 3.3% in patients with four comorbid allergic diseases but it was 1.8% in patients with one allergic disease, and despite the risk of epilepsy was increased by 0.84% in patients with four comorbid allergic diseases, this increase was not significant due to limitation of patients number.

It is undetermined why seizures are associated with the number of comorbid allergic disease in patients. Perhaps multisystem allergic inflammation predisposes children to seizures. On the other hand, since there are different differential diagnoses for

each allergic disease such as asthma, rhinitis, and eczema, and these manifestations can be seen in a wide range of diseases with different pathophysiologies, and the allergic phenotype is only one of the diverse phenotypes of these diseases, co-existence of the several allergic diseases in one person increases the possibility that all these manifestations have occurred in the context of a single genetic background (atopy).

Also, our results showed a significant relationship between epilepsy and the number of allergic diseases in mothers ($p = 0.024$) and not fathers, which suggests the connection between the genetic background of allergies, especially from the mother's side, with the occurrence of idiopathic epilepsy in children.

On the other hand, we found a highly significant relationship between the frequency of febrile convulsion and the number of comorbid allergic diseases in the patients ($p = 0.013$). Because respiratory allergies are considered a risk factor for frequent and febrile respiratory infections due to allergic secretions and inflammation and partial obstruction in some parts of the airways, it is predictable that the prevalence of febrile convulsion in the population of patients with respiratory allergies be higher; but the fact that febrile convulsion are related to the number of allergic diseases in patients indicates that there is a relationship beyond the mentioned case between these two diseases, and maybe the same genetic or inflammatory background is the cause of the emergence and occurrence of these two different diseases in this population of patients.

In addition, in another large retrospective study based on the data of the Taiwan National Health Insurance Research Database, more than 67,000 children with allergic rhinitis and 67,000 people in the control group were compared in terms of the risk of developing epilepsy during a 12-year period and concluded that a group who had allergic rhinitis have a higher incidence of epilepsy compared to the control group (6.84 people *vs* 3.94 people per 10,000 people per year, $p < 0.001$) and the age of onset of epilepsy in this group was lower than the control group, and in the total incidence allergic rhinitis increases the risk of epilepsy by 76% in children, and the risk of epilepsy in boys is 21% higher than in girls. In our study, out of 173 patients with allergic rhinitis, 15 patients had a history of seizures, and 4 patients had a history of idiopathic epilepsy. The prevalence is equal to 23 people per 1,000 people, which although the prevalence is very high, but due to the small size of the sample, it cannot be discussed, and studies with a higher sample size are needed for a more accurate interpretation of the issue.

Finally, it is recommended to conduct more studies with larger sample size in this field, because the most important limitation of the present study was the limitation of the sample size and the lack of integrated information of patients in the Iranian health system.

The limitations of the study are the limited population, no control group, no record of history of atopic diseases in patients; the strengths of the study are the correct and accurate selection of patients based on the physical examination and history taken by asthma and allergy specialists and not on the parents' self-report; attention to the family history of atopy and the division of subgroups of common childhood seizures and determining the relationship of each of these subgroups with the context atopy and not just an allergic disease.

In conclusion, the findings of the current study demonstrated that number of comorbid allergic diseases in the patients is more related to development of some types of seizures than the type of allergic disease and the number of allergic diseases of mothers is related to idiopathic epilepsy in their children.

Fundings

None.

Contributions

HS: conceptualization, investigation, project administration, resources, supervision, validation, visualization, writing – original draft, writing – review & editing. AM: investigation, writing – original draft, writing – review & editing. NT: investigation, data curation, writing – original draft. MB: formal analysis, writing – review & editing. HME: methodology, validation, writing – review & editing. EZ: methodology, writing – review & editing. AZ: validation, writing – review & editing. MT: investigation, resources, supervision, writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Lukas Joerg[1], Nicole Mueller-Wirth[2], Kevin Kammermann[2], Odile Stalder[3], Werner Pichler[2], Oliver Hausmann[2,4]

Quantity increase and functional affinity/avidity decrease of anti-FcεRI and anti-IgE autoantibodies in chronic spontaneous urticaria

[1]Division of Allergology and Clinical Immunology, Department of Pulmonary Medicine, Allergology and Clinical Immunology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland
[2]ADR-AC GmbH, Adverse Drug Reactions, Analysis and Consulting, Bern, Switzerland
[3]CTU Bern, University of Bern, Switzerland
[4]Löwenpraxis Luzern, Lucerne, Switzerland

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Corresponding author
Lukas Joerg
Division of Allergology and Clinical Immunology
Department of Pneumology and Allergology
Inselspital
Bern University Hospital
Freiburgstrasse 20
3010 Bern, Switzerland
ORCID: 0000-0003-1512-4279
E-mail: lukas.joerg@insel.ch

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Impact statement

The quantity and avidity of anti-FcεRI and anti-IgE autoantibodies are dependent on the time of analysis in CSU and are therefore of limited use for the diagnosis of autoimmune forms of CSU.

Summary

Background. *Patients with autoimmune forms of chronic spontaneous urticaria (aiCSU) exhibit autoantibodies against the high-affinity IgE receptor (FcεRI) and IgE. As the presence of these autoantibodies does not correlate with disease activity, the functional affinity/avidity may be relevant in aiCSU. This exploratory study aimed to characterize the quantity and avidity of autoantibodies against IgE and FcεRI over 6 months.* ***Methods.*** *The serum of 49 patients with CSU and 30 healthy control subjects was obtained at baseline and 6 months. Serum was analyzed by ELISA, to determine the quantity and avidity of anti-IgE and anti-FcεRI autoantibodies, and by basophil activation test (CU-BAT).* ***Results.*** *An increase in the quantity of anti-FcεRI and anti-IgE antibodies and a simultaneous decrease in avidity was found in all patients with CSU after 6 months: median anti-IgE increased from 6.7 ng/mL (IQR 5.1-12.5) to 23.8 ng/mL (IQR 12.3-121.5), $p < 0.001$, median anti-FcεRI from 52.4 ng/mL (IQR 26.3-111.4) to 129.5 ng/mL (IQR 73.7-253.7), $p < 0.001$. Median anti-IgE avidity decreased from 75.8% (IQR 55.3-90.8) to 56.4% (IQR 30.6-76.2), $p = 0.019$ and median anti-FcεRI avidity from 75.1% (IQR 49.8-90.0) to 52.2 (IQR 38.2-60.1), $p < 0.001$. In contrast, the frequency of activated basophils did not change significantly over time. Surprisingly, autoantibody avidity did not correlate with basophil activation.* ***Conclusions.*** *Both the quantity and avidity of anti-FcεRI and anti-IgE antibodies change over time, demonstrating that the CU-BAT is more suitable to diagnose aiCSU. In addition, the avidity of anti-FcεRI and anti-IgE antibodies do not correlate with CU-BAT and disease activity, suggesting that further factors independent of anti-FcεRI and anti-IgE autoantibodies contribute to aiCSU.*

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Introduction

Chronic spontaneous urticaria (CSU) is a common skin disease characterized by redness, itchy hives, and angioedema lasting at least 6 weeks (1). Although the exact pathogenesis of CSU is not completely understood, autoimmunity has been proposed in a proportion of patients based on the presence of autoreactive serum components and mast cell activation, as demonstrated by the autologous serum skin test (ASST) (2). The basophil activation test (CU-BAT) was recently established as a specific, sensitive, and safe substitute for the ASST (3).

Approximately one-third to one-half of patients with CSU have IgG autoantibodies directed against IgE or the high-affinity IgE receptor, FcεRI (4). A study also identified IgA and IgM autoantibodies against FcεRI in some patients with CSU (5). It is hypothesized that anti-IgE and anti-FcεRI antibodies play a major role in the pathogenesis of some forms of CSU by activating the FcεRI pathway. Therefore, patients with CSU with a positive ASST/BAT and/or anti-IgE and anti-FcεRI antibodies are subclassified as autoimmune CSU type II (aiCSU) (6). Another CSU subtype is driven by a type I IgE-mediated autoallergy mechanism (aaCSU) and should be distinguished from aiCSU (6). Instead of IgG autoantibodies, patients with aaCSU exhibit IgE autoantibodies against thyroperoxidase, double-stranded DNA, interleukin-24, tissue factor, and thyroglobulin (7-10).

Interestingly, autoantibodies against IgE and FcεRI can be found in patients with other autoimmune diseases and even in healthy individuals (11). Furthermore, autoantibody levels in CSU patients do not change significantly over time, despite undulating disease activity (12). This suggests that these autoantibodies may not be functional. One possibility could be that a second signal or primer, in addition to anti-IgE/anti-FcεRI autoantibodies, is required to trigger basophil/mast cell activation in aiCSU (13). Alternatively, the affinity/avidity of the autoantibodies may be higher in patients with aiCSU due to somatic hypermutation (14). While somatic hypermutation and high-affinity antibodies are beneficial in an immune response to infectious pathogens, high-affinity autoantibodies can exacerbate disease activity in autoimmune diseases (15). The majority of studies focus on the affinity/avidity of antibodies against infectious pathogens (16-18). However, autoantibodies have been better characterized in recent years, especially antiphospholipid antibodies and those against nervous tissue (19, 20). Therefore, determining the quantity and avidity of the anti-IgE and anti-FcεRI autoantibodies in CSU could be of prognostic importance.

This study aimed to characterize autoantibodies against IgE and FcεRI in patients with CSU over 6 months and analyze the correlation between autoantibody avidity and basophil activation.

Materials and methods

Study design and patients

This monocentric, prospective exploratory study investigated the quantity and avidity of autoantibodies against IgE and the FcεRI receptor in CSU patients between January 2018 to December 2019. 49 patients with CSU referred to our allergy unit for an allergologic workup were included in this study. All patients had two study visits with a time interval of 6 months, in which CU-BAT, ELISA, and the urticaria control test (UCT) were performed. All patients included gave informed consent. The study was approved by the local Ethics Committee (Kantonale Ethikkommission Bern: 187/11) and funded by the Ulrich Müller Gierok Allergy Foundation Bern, Switzerland.

Patients between the ages of 18 and 80 with CSU were included in the study if they had symptoms for at least 6 weeks, with hives present at least three times weekly. Patients with inducible or allergic forms of urticaria or documented prior treatment with systemic immunosuppressive agents (except short-term prednisolone under 7 days for CSU exacerbations with a maximal dose of 50 mg prednisolone) were excluded from this study. For comparison purposes, all baseline study procedures were performed in 30 healthy subjects.

Basophil activation test (CU-BAT)

CU-BAT was performed at baseline and at 6 months as previously described (3, 21). Briefly, peripheral mononuclear cells from 2 healthy donors were isolated by density gradient centrifugation, suspended in a test solution (RPMI-1640, 1 mmol/L CaCl2, 1 mmol/L MgCl2, and 1% BSA) and primed with IL-3 (1 ng/mL). From this solution, 100 µL were incubated for 30 minutes at 37 °C with 100 µL of CSU serum. An anti-IgE antibody (100 ng/mL; Beckman Coulter, Marseille, France, Clone E124.2.9), N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) (Sigma-Aldrich, St. Louis, USA), a serum mix of healthy donors (n = 5), and the test solution acted as positive and negative controls. The cells were stained with phycoerythrin conjugated anti-CCR3 (BioLegend, San Diego, USA; clone 5E8) and anti-CD63-FITC (BioLegend, San Diego, USA; clone H5C6) for 20 minutes at 4 °C, then washed with CellWASH. Basophils were detected by CCR3 expression using a FACSCanto flow cytometer. CD63 served as an activation marker and was expressed as the percentage of activated basophils (mean of two measurements).

Basophil characteristics of healthy donors were examined in line with our prior publication by Gentinetta *et al.* (3). In short, healthy donor basophils were tested for their capability to react to different known CU-BAT serum samples as positive control and a serum mix of healthy donors as negative control with gradually increasing IL-3 concentrations to standardize the results across various basophil donors. Overall, slight variations in

basophil reactivity were observed among different donors, but using individual IL-3 concentrations for the different donors these differences were no longer statistically significant and did not correlate with IgE receptor density, as previously noted by Gentinetta *et al*. (3). In addition, the expression of FcεRIα on basophils is mainly regulated by levels of free IgE, which may vary in response to factors such as allergen exposure, smoking, or other environmental influences (22).

Quantity and avidity of anti-IgE and anti-FcεRI autoantibodies

IgG antibodies against FcεRIα and IgE were evaluated using an ELISA at baseline and 6 months. The ELISA plate was coated overnight with appropriate concentrations of the alpha subunit of FcεRI (Human FceRI/FceR1A Protein, Lubio Science, Zurich, Switzerland; concentration of 0.73 µg/ml) or human IgE (human IgE, NBS-C Bioscience, Vienna, Austria; clone SUS-11; 0.5 µg/ml). The plate was blocked with 3% milk PBS for two hours at room temperature after washing (PBS+0.05% Tween20) to reduce non-specific binding. A human anti-FcεRI standard (Creative Biolabs, Shirley, USA; Clone NPB311), humanized anti-IgE (omalizumab, Xolair®, Novartis Pharma AG Basel, Switzerland), and the patient serum were diluted with 1% milk PBS, added to the plate in replicates, and incubated for one hour at 37 °C. The samples were then incubated (1h, 37 °C, 5% CO_2) with the detection antibody, a peroxidase-coupled anti-human IgG antibody (Binding Site, Birmingham, UK; AP004), and developed by adding tetramethylbenzidine (TMB) substrate. The development reaction was stopped with sulfuric acid. The absorbance was measured at 450 nm, and the number of antibodies was determined from the standard curve. For the affinity/avidity analysis, half of the replicates of each patient's serum were incubated with chaotropic reagent ammonium thiocyanate (0.5M), and the other half with 1% milk PBS for 10 minutes, shaking at room temperature at 300rpm. Prior to the assay, different concentrations of ammonium thiocyanate (0-1.5M) were tested, and for subsequent testing, a concentration of 0.5M was used. After an immediate washing step, the samples were incubated (1h, 37 °C, 5% CO_2) with the detection antibody and developed by adding TMB substrate. The absorbance was measured at 450 nm. The avidity of the autoantibody was expressed as the percent residual autoantibody quantity at 0.5 M of the chaotropic reagent.

Urticaria Control Test (UCT)

UCT scores were measured at baseline and 6 months. The UCT is a validated, retrospective questionnaire with 4 questions to assess disease activity in patients with chronic urticaria during the previous month (23). Each question can be answered with 5 options, scored with 0 to 4 points. The cumulative minimum scores range from 0 to 16, with 16 indicating complete urticaria control.

Post-hoc analysis

A subset of study participants was selected for an additional avidity evaluation by surface plasmon resonance assay (SPR) testing (Biacore, Cytiva, Uppsala, Sweden) to compare with the ELISA assay and to provide additional insights into binding interactions. This subset was chosen based on the strength of binding interactions observed with chaotropic reagents and the availability of samples. Only baseline samples were evaluated.

Surface plasmon resonance measurements

All SPR measurements were performed on a GE Healthcare Biacore X100 device (IL, USA). HBS-EP+ was used as a running buffer at a 10 µL/min flow rate. Recombinant human FcεRIα were immobilized on flow cell 2 (Fc2) of a CM5 sensor chip by standard amine coupling at a target level of 1,000 RU. A blank immobilization was performed with the reference flow cell 1 (Fc1). Binding responses were displayed as Fc2 signals minus binding responses on the Fc1 reference cell. Recombinant human IgE (Sus11-IgE, NBS-C BioScience) at a concentration 20 nM was captured on the immobilized FcεRIα for 120 seconds. Different dilutions (*i.e.*, 1:2, 1:4, 1:8 1:16 and 1:32) of purified IgG fractions (NAb™ Protein G Spin Kit, Thermo Scientifc) from CSU patient sera were injected for 120 seconds with a dissociation time of 180 seconds between each injection under constant buffer flow on the pre-formed IgE:FcεRIα complexes. At the end of each run the chip surface was regenerated for 14 seconds with 25 mM NaOH and reloaded with Sus11-IgE. To determine binding kinetics, we used the BIAevaluation software. Affinity constants were calculated using a 1:1 Langmuir curve fitting model.

Statistical analysis

Baseline characteristics between patients and control participants were compared using a Chi-squared test or non-parametric Wilcoxon rank-sum test, as appropriate. A Wilcoxon signed-rank test was used to compare the patients with CSU at baseline and 6 months. Associations between CU-BAT and autoantibody avidity were calculated using Spearman's rho. For subgroup analysis, all patients with elevated autoantibody titers at baseline were analyzed independently. Cut-offs for subgroup analysis of antibody quantity were determined as the mean plus twice the standard deviation of the control group. Stata 16 (Stata Corporation, College Station, Texas, USA) was used for all statistical analyses, and P-values less than 0.05 were considered statistically significant. Some patients had to start omalizumab treatment before the 6 months follow-up. All the measurements related to quantity and avidity measurements of anti-IgE antibodies (but not CU-BAT or anti- FcεRI) were ignored if the patient was under omalizumab medication due to its influence: omalizumab was the standard of this assay. Associations between autoantibody avidity by chaotropic agents and surface

plasmon resonance assay (SPR) were calculated using Spearman's rho.

Results

Study patients

A total of 49 patients with CSU and 30 healthy subjects were included in this study (**table I**). The proportion of males was 29% and 33% in patients with CSU and healthy subjects, respectively. The median age was 35.0 (IQR 25.0-49.0) in patients with CSU, which was slightly higher compared to healthy controls (26.5 (IQR 23.0-39.5); p = 0.05). The median disease duration at baseline was short at 7.0 (IQR 4.0-15.0) months. At baseline, 57% of patients with CSU had angioedema, 94% were on antihistamines, 4% on montelukast, 10% on systemic steroids in the 6 months before (1 patient with 40 mg prednisolone 6 month, two patients with 50 mg 2 and 4 months and two with 20 mg prednisolone 1 month prior to inclusion, no patients under systemic steroids at inclusion), and one patient was on omalizumab. At 6 months, 8 patients were under omalizumab treatment, none of the patients had systemic steroid treatment. The median UCT score at baseline was 6.0 (IQR 3.0-8.0) and was significantly higher after 6 months (12.0; IQR 11.0-14.5; p < 0.001), indicating lower CSU activity. CSU had subsided in 5 subjects at the 6-month follow-up.

Baseline comparison of the quantity and avidity of anti-FcεRI and anti-IgE autoantibodies and basophil activation

We first compared the quantity and avidity of anti-FcεRI and anti-IgE autoantibodies in patients with CSU and healthy controls at baseline (**table I**). The median quantity of anti-IgE was not significantly different in the CSU group (6.7 ng/mL; IQR 5.1-12.8) compared to the control group (9.6 ng/mL (4.9-28.3); p = 0.377). Similarly, the median quantity of anti-FcεRI was not significantly different (52.4 ng/mL; IQR 25.7-126.5 *vs* 49.3 ng/mL; IQR 29.8-177.1; p = 0.649). The median avidity of anti-IgE was 75.8% (IQR 55.0-91.8), and the median avidity of anti-FcεRI was 75.0% (48.8; 90.1), which also did not differ from the control group (p = 0.951 and p = 0.425, respectively).

Table I *- Patient characteristics and comparison of CSU patients and controls at baseline.*

	All (n = 79)	Patient (n = 49)	Control (n = 30)	P-value
	n (%) or median (IQ-range)	n (%) or median (IQ-range)	n (%) or median (IQ-range)	
Gender (male)	24 (30%)	14 (29%)	10 (33%)	0.655
Age (years)	31.0 (24.0-48.0)	35.0 (25.0-49.0)	26.5 (23.0-39.5)	0.050
Duration of disease (months)	31.0 (24.0-48.0)	7.0 (4.0-15.0)	-	-
Angioedema (yes)	31.0 (24.0-48.0)	28 (57%)	-	-
Antihistamines (yes)	31.0 (24.0-48.0)	46 (94%)	-	-
Montelukast (yes)	31.0 (24.0-48.0)	2 (4%)	-	-
Corticosteroids (yes)	31.0 (24.0-48.0)	5 (10%)	-	-
Omalizumab (yes)	31.0 (24.0-48.0)	1 (2%)	-	-
UCT	10.0 (6.0-16.0)	6.0 (3.0-8.0)	16.0 (16.0-16.0)	< 0.001
anti-IgE quantity ng/mL	7.5 (5.0-13.5)	6.7 (5.1-12.8)	9.6 (4.9-28.3)	0.377
anti-IgE Avidity %	72.7 (51.9-92.9)	75.8 (55.0-91.8)	68.1 (49.1-94.7)	0.951
anti-FcεRI quantity ng/mL	50.0 (26.8-141.6)	52.4 (25.7-126.5)	49.3 (29.8-177.1)	0.649
anti-FcεRI Avidity %	76.2 (53.3-90.2)	75.0 (48.8-90.1)	79.6 (54.7-91.2)	0.425
CU-BAT	1.3 (0.6-3.8)	2.8 (1.2-4.9)	0.7 (0.5-1.1)	< 0.001
CU-BAT heated	2.8 (1.9-3.6)	3.0 (2.0-4.4)	2.4 (1.5-3.2)	0.008
CU-BAT > 7.6% (yes)	7 (9%)	7 (14%)	0 (0%)	0.040
CU-BAT heated > 7.6% (yes)	10 (13%)	10 (20%)	0 (0%)	0.011
anti-IgE quantity > control mean + 2sd	18 (23%)	8 (16%)	10 (33%)	0.104
anti-FcεRI quantity > control mean + 2sd	24 (30%)	15 (31%)	9 (30%)	1.000

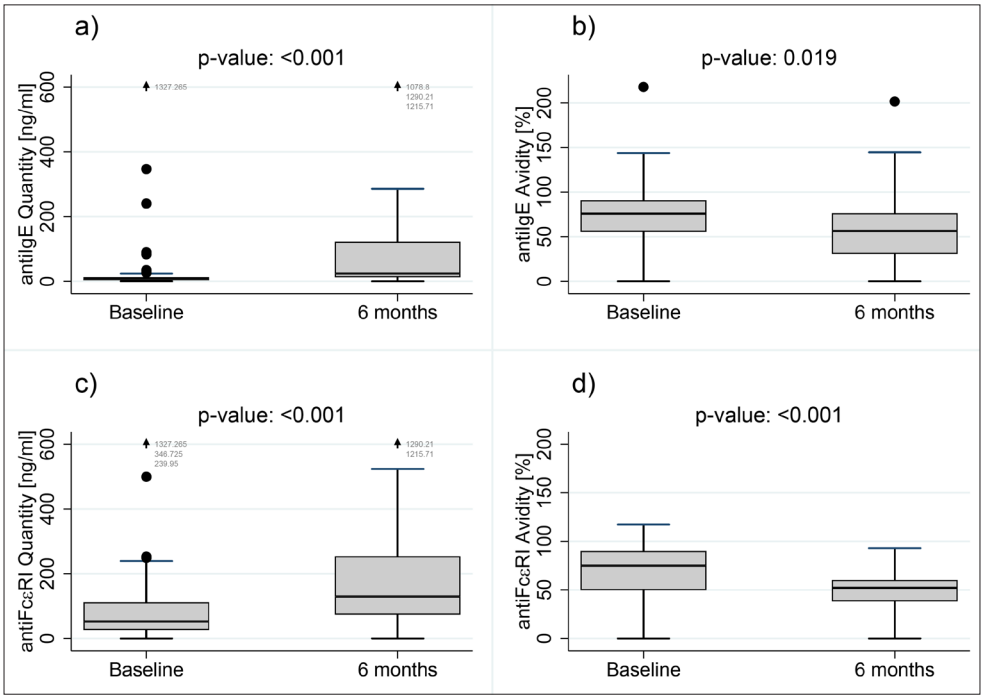
Next, we used the CU-BAT to investigate whether basophil activation differed between patients with CSU and healthy controls (**table I**). In contrast to the quantity and affinity of autoantibodies, the median frequency of activated basophils was significantly higher in the CSU group compared to the control group (2.8% (IQR 1.2-4.9) *vs* 0.7% (IQR 0.5-1.1); p < 0.001). According to the literature, we set the cut-off for CU-BAT positivity at > 7.6% as an indication of aiCSU (3). We observed a positive CU-BAT in 7 patients with CSU (14%) and none of the control group using this cut-off. To determine whether immunoglobulins are sufficient for basophil activation, we heat-inactivated the serum to degrade all heat-labile factors and preserve immunoglobulins. Both the frequency of activated basophils and subjects with a positive CU-BAT remained significantly higher in patients with CSU, indicating that antibodies are required for the enhanced basophil activation seen in patients with CSU.

Comparison of autoantibodies and basophil activation in patients with CSU at baseline and 6 months

To determine whether autoantibodies change over time in patients with CSU, we compared the quantity and avidity of autoantibodies and frequency of basophil activation at baseline and the 6-month follow-up. At 6 months, the median quantity of anti-IgE increased from 6.7 ng/mL (IQR 5.1-12.5) to 23.8 ng/mL (IQR 12.3-121.5; p < 0.001), and the quantity of anti-FcεRI increased from 52.4 ng/mL (IQR 26.3-11.4) to 129.5 ng/mL (IQR 73.7-253.7; p < 0.001) (**figure 1**). In contrast, the median avidity of anti-IgE decreased from 75.8% (IQR 55.3-90.7) to 56.4% (IQR 30.6-76.2; p = 0.019), and the avidity of anti-FcεRI decreased from 75.1% (IQR 49.8-90.0) to 52.2% (IQR 38.2-60.1; p < 0.001) after 6 months (**figure 1**). Interestingly, the median frequency of activated basophils did not differ significantly after 6 months (2.8% (IQR 1.2-4.9) to 1.8% (IQR 0.5-4.3; p = 0.805) (**figure 2**). The frequency of CU-BAT-positive patients decreased after 6 months but was not statistically significant.

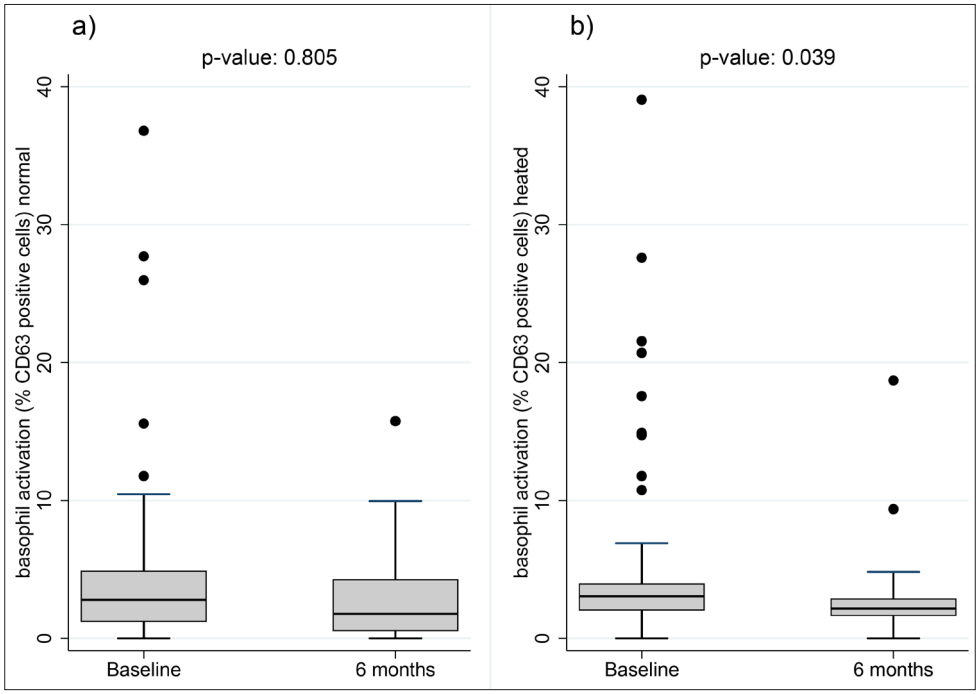
We next compared the baseline and 6-month timepoints of patients with elevated levels of anti-IgE or anti-FcεRI autoantibodies (**table II**). Patients were classified as having elevated autoantibodies if the autoantibody quantity was higher than the mean plus twice the standard deviation of the control group at baseline. In these patient subgroups, the quantity of anti-IgE and anti-FcεRI also increased while the avidity decreased. However, the difference in autoantibody quantity was not statistically significant. We saw similar results in patients where CSU subsided after 6 months (**figure 1S**). There were no significant differences in the frequency of activated basophils at baseline and 6 months in either subgroup (**table II**).

Figure 1 *- Quantity and avidity of anti-FcεRI (n = 30) and anti-IgE autoantibodies (n = 22) in patients with CSU at baseline and the 6-month follow-up.*



IgG antibodies against FcεRIα and IgE were evaluated for quantity ***(a, c)*** *and avidity* ***(b, d)*** *using an ELISA at baseline and 6 months. Data represent the median and interquartile range at each timepoint. P-values were determined using a Wilcoxon signed-rank test. Outliers which lie out of the graph range are indicated with an arrow. Only the patients with baseline and 6 months measurements were considered for the computation.*

Figure 2 - *The frequency of activated basophils in patients with CSU at baseline and the 6-month follow-up (n = 30).*



The serum of patients with CSU at baseline and 6 months were incubated with basophils isolated from 2 healthy donors (CU-BAT) to determine the presence of activating serum factors ***(a)****. CU-BAT analysis of heat-inactivated serum* ***(b)****. CD63 served as an activation marker and was expressed as the percentage of activated basophils (mean of two measurements). Data represent the median and interquartile range at each timepoint. P-values were determined using a Wilcoxon signed-rank test.*

Correlation of autoantibody avidity with the frequency of activated basophils

We next determined whether the frequency of activated basophils correlated with anti-IgE and anti-FcεRI avidity. Surprisingly, there was no significant correlation between the frequency of activated basophils and autoantibody avidity (**figure 3**). Similar results were seen in the subgroup of patients with elevated autoantibodies at baseline (data not shown).

CSU activity

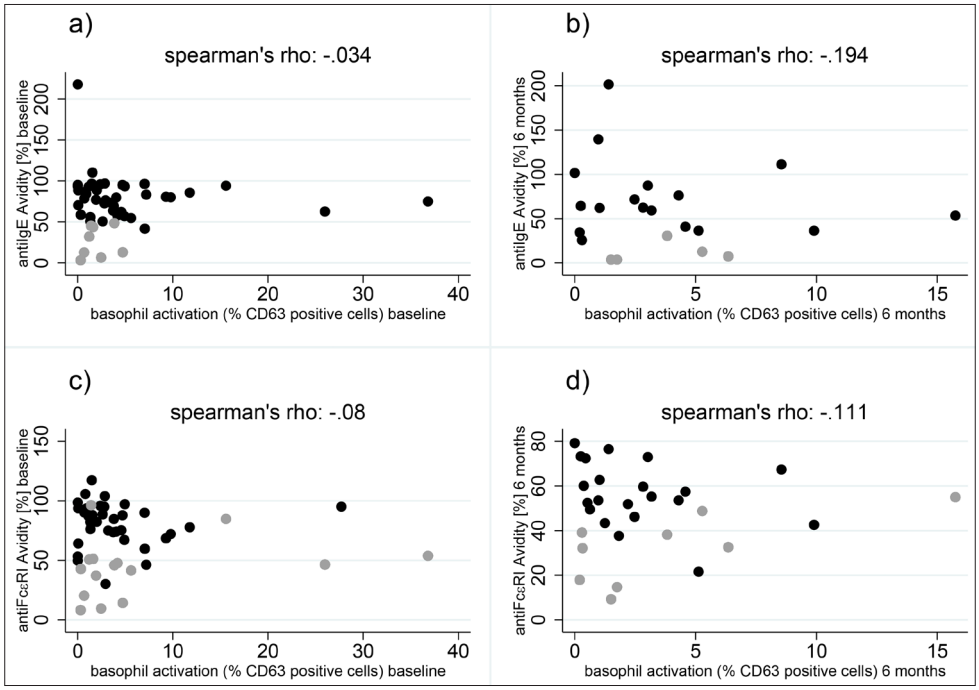
CSU activity was calculated based on the UCT score. After 6 months, there was a significant increase, which means a lower

Table II - *Subgroup analysis of patients with elevated levels of anti-FcεRI (n = 9) and anti-IgE (n = 5) at baseline.*

	Baseline	6 months	P-value
	Median (IQ-range)	Median (IQ-range)	
Anti-IgE quantity subgroup			
Anti-IgE quantity (ng/mL)	86.5 (30.5-293.3)	1,078.8 (34.4-1215.7)	0.138
Anti-IgE Avidity %	22.6 (9.7-44.5)	7.4 (3.7-12.7)	0.043
CU-BAT	1.5 (0.9-3.1)	2.8 (1.5-5.3)	0.463
Anti-FcεRI quantity subgroup			
Anti-FcεRI quantity (ng/mL)	151.6 (141.6-499.4)	260.8 (162.4-464.8)	0.173
Anti-FcεRI avidity %	45.9 (20.4-51.2)	32.6 (17.9-39.1)	0.051
CU-BAT	2.5 (1.2-5.6)	1.8 (0.3-5.3)	0.594

All patients with elevated autoantibody titers at baseline were analyzed independently for subgroup analysis. Cut-offs for subgroup analysis were determined as the mean plus twice the standard deviation of the control group. P-values were calculated by the Wilcoxon signed-rank test.

Figure 3 - *Correlation of the frequency of activated basophils with the avidity of anti-FcεRI and anti-IgE autoantibodies in patients with CSU.*



*Association of the frequency of activated basophils, as determined by a basophil activation test, and the avidity of anti-IgE (**(a)** n = 48; **(b)** n = 22) and anti-FcεRI (**(c)** n = 49; **(d)** n = 30) autoantibodies (%) at baseline and 6 months. The strength of association was calculated by Spearman's rho. Patients with autoantibody quantities above the cut-off value at baseline are plotted in grey.*

CSU activity. The median UCT score at baseline was 6.0 (3.0; 8.0) and at 6 months 12.0 (11.0; 14.5); p < 0.001.

Post-hoc analysis

We evaluated a subset of study participants (11 CSU Patients and 6 healthy controls) at baseline with a second avidity assay by SPR. The avidity was measured by the equilibrium dissociation constant (KD). The median KD value at baseline for CSU patients was 0.170 [0.065; 0.336] M/1,000,000 and 0.029 [0.022; 0.232] M/1,000,000 for healthy controls, p = 0.16. We compared the SPR avidity values to the ELISA avidity and calculated Spearman's rho. We found a moderate to a strong positive association between the two assays for the avidity of anti-IgE (Spearman's rho +0.65, p = 0.031) and a strong association for the avidity of anti-FcεRI (Spearman's rho +0.73, p = 0.014).

Subgroup analysis with exclusion of patients under omalizumab and with systemic steroids prior to inclusion

Even after exclusion of all patients on omalizumab and those on systemic steroids before study inclusion, there was an increase in the quantity of anti-IgE and anti-FcεRI and a decrease in avidity. We found no relevant differences of the primary endpoint after exclusion of these patients. Details of this subgroup analysis are shown in **table III**.

Discussion and conclusions

In this study, we found that the quantity of anti-IgE and anti-FcεRI autoantibodies increased, while the avidity decreased over 6 months in patients with CSU, independent of disease course. In contrast, the frequency of activated basophils did not change over time. Unlike previous studies (4), we found signs of aiCSU (based on a positive CU-BAT) in only 14% of patients with CSU. Consistent with our study, MacGlashan *et al*. also demonstrated a lower frequency of aiCSU (24). Although aiCSU is associated with the presence of anti-FcεRI and anti-IgE autoantibodies, not all patients with a positive CU-BAT had elevated autoantibodies, and the quantity significantly changed over 6 months. Our data suggest that the amount of anti-FcεRI and anti-IgE autoantibodies is dependent on the time of analysis, does not correlate to disease activity and is therefore not suitable for the diagnosis of aiCSU.

Our data did not support our hypothesis of a correlation between autoantibody avidity and the frequency of activated basophils, not even in the subgroup analysis of patients with elevated autoantibodies at baseline. Interestingly, the quantity

Table III - *Subgroup analysis of patients without omalizumab or systemic steroids (n = 21).*

	Baseline	6 months	P-value
	Median (IQ-range)	Median (IQ-range)	
Anti-IgE Quantity subgroup			
Anti-IgE quantity (ng/mL)	7.1 (5.3-12.4)	25.6 (12.3-121.5)	< 0.001
Anti-IgE Avidity %	76.1 (54.7-88.7)	53.4 (30.6-76.2)	0.027
Anti-FcεRI quantity subgroup			
Anti-FcεRI quantity (ng/mL)	68.7 (26.7-143.0)	153.4 (83.8-260.8)	0.002
Anti-FcεRI avidity %	75.2 (47.7-90.2)	53.6 (38.2-62.7)	0.001
CU-BAT	2.4 (0.8-4.7)	2.8 (1.0-5.1)	0.198

IgG antibodies against FcεRIα and IgE were evaluated for quantity and avidity using an ELISA at baseline and 6 months. Data represent the median and interquartile range at each timepoint. P-values were determined using a Wilcoxon signed-rank test. Only the patients with baseline and 6 months measurements were considered for the computation.

and avidity of both autoantibodies did not differ significantly between CSU patients and healthy controls at baseline. Healthy subjects may have non-functional anti-FcεRI belonging to the IgG2 subclass, which are unable to activate mast cells/basophils (25). An additional explanation for this finding might be that autoantibodies play only a partial role in aiCSU and that a second signal is required (13). Indeed, numerous other factors like autoantibodies such as IgG anti-thyroperoxidase or IgE antibodies against autoantigens, which we did not measure in this study, and complement play an important role (26, 27). Several studies have shown that the coagulation cascade as well as the complement system, in particular C5a is relevant in the pathogenesis of CSU, both as an augmentation factor related to autoantibodies, but also independently (27-29). Furthermore, we also performed the CU-BAT with heat-inactivated serum to determine the direct influence of immunoglobulins and did not observe a significant correlation. As the avidity measurement by ELISA with chaotropic reagents is not standardized in CSU and a novel approach, we compared a subset of baseline participants with an alternative approach by SPR and found for anti-IgE a moderate to strong and for the avidity of anti-FcεRI a strong association between the two assays.

The increase of autoantibody quantity and the simultaneous decrease of avidity could be attributed to excess and constant antigen exposure, which may impair further affinity maturation, resulting in an enhanced number of low avidity antibodies as observed in patients with infectious diseases (30). Alternatively, there may be a trade-off between high affinity and stability (31). Interestingly, we observed the same autoantibody dynamics in patients with CSU treated with omalizumab, suggesting high disease activity and milder disease, including those with subsided CSU. Our data demonstrate a decrease in the UCT score after 6 months; however, this may have been influenced by medications and not a reflection of actual disease activity. We suspect that the autoantibody changes occur in all patients with CSU over time, regardless of disease progression. Interestingly, basophil activation does not appear to be dependent on this mechanism.

A weakness of our study is the inclusion of all CSU cases, regardless of phenotype. Only a fraction of our patients qualified as aiCSU based on a positive CU-BAT result or elevated autoantibodies, which hampered the assessment of autoantibody dynamics. However, the subgroup of study participants with autoantibody titers over the cut-off level showed similar results. In further studies, aiCSU patients should be pre-selected, *e.g.*, with the inclusion of CU-BAT-positive individuals only.

The avidity evaluation in CSU by ELISA via chaotropic reagents is a new approach. To substantiate the results, we performed a *post-hoc* analysis with SPR as a different avidity analysis to compare the results of avidity measurements. Another limitation is the assessment of the UCT score without considering individual drug intake. Drug therapy was very heterogeneous and changed significantly in most patients during the 6-months observation period, resulting in a significant reduction in the UCT. Therefore, a conclusive comparison of the UCT score to CU-BAT and antibody results was not possible. In addition, some patients received omalizumab treatment during the study and had short courses of systemic steroids prior to inclusion. Especially omalizumab influenced the analysis of anti-IgE antibodies. Therefore, we omitted the 6-months measurements of anti-IgE values in patients under omalizumab. An influence on other laboratory values (*e.g.*, IgG values under systemic steroids) cannot be excluded. However, systemic steroids were used only for short courses with a dose of maximal 50 mg prednisolone. In addition, omalizumab does not appear to have an effect on IgG (32). A subgroup analysis excluding these patients as well as those with a history of systemic steroids showed no significant changes of the primary endpoints.

Our study shows that aiCSU patients are significantly less frequent than expected. This may be related to a certain selection bias in previous studies, especially since severe and longtime CSU courses are increasingly treated in large central hospitals. In future our findings should be examined exclusively in aiCSU or patients with elevated autoantibody titers. A more extended observation period and comparing the avidity with clinical activity (together with the recording of drug intake) would be important.

In this study, in conclusion, we found that in patients with CSU the quantity of anti-FcεRI and anti-IgE autoantibodies increases, and the avidity decreases over time. However, neither the quantity nor the avidity of these autoantibodies correlates with disease course or the frequency of activated basophils, suggesting that factors independent of these two autoantibodies play a significant role in aiCSU. Our data suggest that the quantity and avidity of anti-FcεRI and anti-IgE autoantibodies are dependent on the time of analysis and are therefore not suitable for the diagnosis of aiCSU.

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Contributions

LJ, NMW, OH: conceptualization, methodology, project administration, writing - olriginal draft. LJ: funding acquisition. LJ, NMW, KK, OH: investigation, validation. LJ, OD: data curation, formal analysis. All authors: writing - review & editing.

Conflict of interests

NMW, KK, WP and OH are employees of ADR-AC GmbH, a specialized laboratory offering basophil activation tests for routine diagnostics in Switzerland. Other authors declare that they have no conflict of interests.

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LETTER TO THE EDITOR

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Cláudia Varandas[1], Josefina Vieira[1], Catarina Jacinto Correia[2], Marisa Paulino[1], Amélia Spínola Santos[1,3], Anabela Lopes[1,3], Susana Lopes Da Silva[1,3], Elisa Pedro[1,3], Joana Caiado[1,3]

Hypersensitivity reactions to iron products: 10-year experience in a Portuguese tertiary center

[1]Department of Immunoallergology, Santa Maria Hospital, Centro Hospitalar Universitário Lisboa Norte, Centro Académico de Lisboa, Lisbon, Portugal
[2]Department of Immunohemotherapy, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal
[3]Immunoallergology University Clinic, Faculty of Medicine, Lisbon University, Lisbon, Portugal

Key words

Iron products; hypersensitivity reactions; premedication; re-administration; desensitization.

Corresponding author
Cláudia Varandas
Department of Immunoallergology
Santa Maria Hospital
Centro Hospitalar Universitário Lisboa Norte
Av. Professor Egas Moniz
1649-035 Lisbon, Portugal
ORCID: 0000-0002-8785-4097
E-mail: varandas@campus.ul.pt

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To the Editor,

iron products (IP) have been increasingly used in recent years and constitute the first-line treatment for iron deficiency anemia (1). Although hypersensitivity reactions (HSR) to IP are rare and the risk of anaphylaxis with the currently available iron formulations has been shown to be significantly lower compared to iron dextran (2, 13-15), there is still a concern regarding the safety of these products, with cases of fatal outcome reported in the literature (2, 3). Here, we evaluate the safety of controlled re-administration of IP in patients with previous hypersensitivity reactions to these drugs.

We performed a retrospective review of patients referred to the Immunoallergology Department in a tertiary hospital for suspected HSR to IP, from 2011 to 2021. The demographic and clinical characterization of a total of 18 patients (mean age 45.6 ± 14.3 years) is summarized in **table I**. We observed a higher prevalence of female patients (83%), likely due to the higher frequency of treatment with IP among women (1). Regarding relevant past medical history, six patients (33%) reported previous reaction(s) to non-IP drugs, a similar prevalence found by Steveling-Klein *et al.* in a group of 22 adult patients out of 59 (37%) (4). Three patients were atopic (16%) and two presented with inflammatory diseases (sarcoidosis and inflammatory bowel disease; 11%). Interestingly, only 67% reported previous exposure to IP (**table I**).

The IP most frequently involved in hypersensitivity reactions was ferric carboxymaltose (FCM) in 12 patients (63%), followed by oral non-specified iron formulation in four patients (21%) and iron sucrose (IS) in three (16%). One patient presented HSR to both FCM and IS. The severity of reactions was categorized according to the Ring and Messmer Grading

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***Table I** - Clinical characterization of patients with hypersensitivity reactions to iron products.*

Characteristics	Total (n = 18)
Gender, n (%)	
Female	15 (83.3)
Age, years	
Mean ± SD (minimum - maximum)	45.6 ± 14.3 (15.0-72.0)
Relevant medical history, n (%)	
Non-IP drug allergy	6 (33.3)
Atopy	3 (16.7)
Rhinitis	3 (16.7)
Asthma	1 (5.6)
Sarcoidosis	1 (5.6)
Inflammatory bowel disease	1 (5.6)
Previous exposure to IPs, n (%)	
Exposure without reaction	12 (66.7)
No exposure	6 (33.3)

IP: iron products; n: total number of patients; SD: standard deviation.

Scale for anaphylactic reactions. The majority of the reactions were classified as grade I (n = 11, 58%). Grade II occurred in two HSR (11%) and grade III in five (26%), comparable to the distribution reported in the literature (4). One patient (5%) reported an immediate reaction with cyanosis, dyspnea, hypoxemia and circulatory arrest, categorized as grade IV, two minutes after FCM administration. Interestingly, intravenous IP were associated with increased severity of reactions. Drug allergy, atopy, mastocytosis, severe asthma/eczema, severe cardiac or respiratory disease, fast infusion rate, old age and concomitant systemic inflammatory disease have been identified in the literature as risk factors for increased risk and/or severity of IP hypersensitivity reactions (4, 5). In contrast, patients in our population with atopy did not present significantly different severity compared to non-atopic patients, nor did patients with previous hypersensitivity reactions to non-IP drugs ($p = 0.154$ and $p = 0.086$, respectively; Mann-Whitney Test, Graph-Pad Prism v5.01). Age was also not correlated to severity ($r = -0.19$, $p = 0.44$; Spearman Correlation).

During diagnostic work-up, skin prick and intradermal tests with the suspected and/or alternative IP were performed on 12 patients, with FCM (Ferinject®, 50 mg/mL; n = 10), and/or IS (Venofer®, 20 mg/mL; n = 11). We found a positive result only in one patient (ID test with IS, 100-fold dilution), in agreement with results from other studies on HSR to IP. These results are in line with the most likely putative mechanism of HSR to non-dextran-derived IP: complement activation-related

***Table II** - Detailed characterization of hypersensitivity reactions to iron products.*

Characteristics	Total (n = 18)
Culprit IP, n (%)†	19 (100)
Ferric carboxymaltose	12 (63.2)
Oral IP preparation	4 (21.1)
Iron sucrose	3 (15.8)
Grade of reaction, n (%)*,†	
I	11 (57.9)
Oral IP preparation	4
Ferric carboxymaltose	7
II	2 (10.5)
Iron sucrose	2
III	5 (26.3)
Ferric carboxymaltose	4
Iron sucrose	1
IV	1 (5.3)
Ferric carboxymaltose	1
Positive IP skin tests/total, n†	
Ferric carboxymaltose	0/10
Iron sucrose	1/11
IP re-administration, n	15
Ferric carboxymaltose	5
Premedication	4
Alternative IP/culprit IP	3/2
Tolerated	5
Iron sucrose	10
Premedication	6
Alternative IP/culprit IP	10/0
Tolerated	10
IP desensitization, n†	9
Patient 1	1
Ferric carboxymaltose	1
Premedication	1
Alternative IP/culprit IP	0/1
Tolerated	1
Patient 2	1
Iron sucrose	1
Premedication	1
Alternative IP/culprit IP	0/1
Tolerated	1
Patient 3	7
Ferric carboxymaltose/iron sucrose	1/6
Premedication	7
Alternative IP/culprit IP	0/7
Tolerated	0

n: total number of patients; *according to Ring and Messmer Grading Scale; †more than 1 procedure per patient.

pseudo-allergy (2, 4, 6, 7). In contrast to the rare IgE-mediated HSR, self-limited transitory flushing and truncal myalgias, denominated as Fishbane reactions, are common and often misdiagnosed as hypersensitivity reactions (2). Importantly, in both IgE and non-IgE HSR mechanisms, desensitization has been reported to be effective, although time-consuming (8-12).

In clinical practice we may consider three alternative strategies in the management of patients with HSR to IP: graded re-administration, desensitization, and use of premedication prior to IP.

In our study, the outcomes of these strategies were registered (**table II**). Patients to whom iron was re-administered gave oral and written informed consent.

IP re-administration was successfully conducted in 15 patients (ten IS and five FCM; using premedication, antihistamines ± corticosteroids, in ten patients), in two of them with the culprit IP. IP desensitizations were conducted according to Mariana Castells' 12-step protocol (12) and performed in three patients, (seven IS and two CMF), all with the culprit agent. Desensitization was tolerated by two patients (one IS and one CMF), one of them being the patient with a positive IDT test to IS. The third patient, with a history of severe iron deficiency anemia secondary to inflammatory bowel disease, failed to complete all seven desensitization protocols (six IS and one CMF). She experienced grade III HSR during the tolerance inducing protocols, which prevented her from achieving the total required therapeutic dose.

The scarcity of cases of hypersensitivity reactions to IP, documented during a 10-year activity in an Allergy Clinic from a tertiary hospital, is in agreement with the rare prevalence of these reactions reported in the literature (3). Detailed clinical history and implementation of risk minimization measures, particularly slow infusion rates (iron sucrose 200 mg/3h and ferric carboxymaltose 500 mg/3h), are decisive for the management of hypersensitivity reactions and are mostly sufficient to minimize the occurrence of mild and Fishbane reactions, these latter frequently misdiagnosed as allergic reactions (13).

Our results stand out against the position of the European Medicines Agency, stating that intravenous iron products are contraindicated in patients with known serious hypersensitivity to any parenteral iron product (19). In fact, our study supports recent observations regarding the safety of re-challenge with an alternative IP, and even with the culprit formulation, which are generally well tolerated in mild and moderate reactions (4, 6, 20). Additionally, the absence of HSR upon re-administration of an alternative IP, regardless of the use of premedication (eight with premedication; five without premedication), underlines the difficulty in determining the benefit of premedication. Re-administration of the culprit IP was performed, and tolerated, in two patients, with premedication. Notably, the evidence supporting premedication with antihistamines is uncertain, as their vasoactive effects can rather be misinterpreted as anaphylaxis symptoms, however, this is still recommended as a strategy to minimize the risk of reaction to IP (3, 13, 16-18).

The authors highlight that the implementation of a low-reactogenic administration protocol in clinical practice, consisting of a slow infusion rate, corresponding to an infusion rate three and six times slower than the recommended for ferric carboxymaltose and iron sucrose, respectively, should be encouraged. Recommendations regarding premedication should be addressed in further studies. Patient management should be individually decided according to the severity of the HSR, however, the authors herein report that the majority of patients tolerated the re-administration of an alternative or even the same IP, emphasizing the safety of this approach.

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Contributions

CV, JC: conceptualization, formal analysis, writing - original draft. CV, JC, JV, CJC, MP, ASS, AL, SLS, EP: data curation, writing - review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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LETTER TO THE EDITOR

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Katherine Pose[1], Margarita Tomás-Perez[1,2]

Standard dose paracetamol-induced tubulointerstitial nephritis

[1]Department of Allergy, La Paz University Hospital, Madrid, Spain
[2]Institute for Health Research (IdiPAZ), Madrid, Spain

Key words

Tubulointerstitial nephritis; paracetamol; lymphocyte transformation test; NSAIDs.

Corresponding author
Katherine Pose
Department of Allergy
La Paz University Hospital
P.º de la Castellana 261
Fuencarral-El Pardo, 28046 Madrid, Spain
ORCID: 0000-0002-3421-5304
E-mail: katherine.pose@salud.madrid.org

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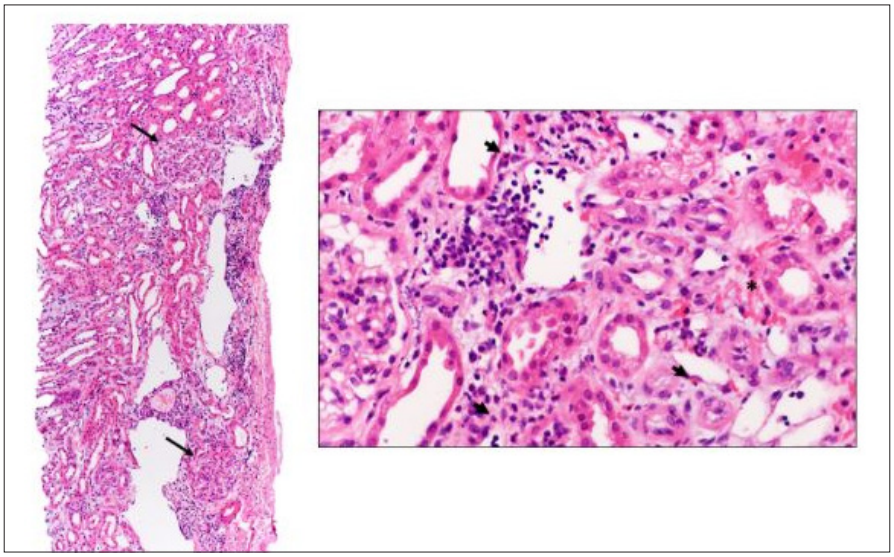
To the Editor,

paracetamol is widely used as an analgesic and antipyretic agent safe for all age groups and even for chronic kidney disease patients, freely prescribed by many physicians and available as an over-the-counter drug at pharmacies (1). Tubulointerstitial nephritis (TIN) is a group of immune-mediated inflammatory diseases that involve the interstitium and tubules. It is one of the most important and major causes of acute kidney injury that subsequently leads to renal failure. Drug-induced nephritis is the leading cause of TIN and its most important etiology is medication such as paracetamol and other NSAIDs (2). Histopathological view after the renal biopsy is the only definitive diagnosis and gold standard for TIN, which shows characteristic diffuse or focal inflammatory cells (2). The basic treatment is supportive therapy, such as body fluid and electrolyte management, discontinuation of drugs is the first line of treatment in drug-induced TIN with alternative drug administration to treat infections that often lead to the reversal of renal injury (2, 3). Although the effectiveness of steroids is controversial, some studies have reported a more rapid and complete recovery of baseline renal function in those patients treated with steroids; its initiation in the early phase has shown efficacy (4). There are just a few cases reported on paracetamol-induced TIN with standard doses (5). None of the previous cases reported were confirmed by a lymphocyte transformation test (LTT). We present a case of acute tubulointerstitial nephritis induced by paracetamol confirmed by LTT.

A 25-year-old woman with a 72-hour bilateral lumbar pain radiating to the flanks, nausea, and vomiting. To relieve the pain she took 1 gram of paracetamol every 8 hours (previously tolerated without incidence), with a total of 5 pills taken in that time. Due to worsening symptoms, she went to the emergency room where she was finally admitted by Nephrology due to a non-oliguric AKIN 3 acute renal failure with a non-anion gap metabolic acidosis. Laboratory tests showed creatinine 6.05 mg/dL, urea 64 mg/dL, eosinophils 90/µL, and leukocytosis of 12,770/µL. Glomerular filtration (CDK-EPI) 12 mL/min/1.73 m^2. The anion gap was 12. Urinary analysis showed sodium 61 mmol/L, chloride 50 mmol/L, potassium 23 mmol/L, and leukocyturia. Obstructive etiology was ruled out with the abdominal ultrasound, and the renal biopsy revealed tubulointerstitial nephritis, nephrocalcinosis, and tubular necrosis (**figure 1**).

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Figure 1 *- Patient's kidney biopsy findings.*



Patchy areas of interstitial edema and mixed inflammatory infiltrates with lymphocytes, neutrophils, and occasional eosinophils (arrowheads) are recognized. There are regenerative changes and vacuolization in the proximal convoluted tubules and foci of interstitial microhemorrhage (asterisk). Images of tubulitis are not identified. The glomeruli have fine and permeable capillary lumens and do not show significant histological alterations (arrows).

She was then assessed by the department of allergology and we performed an LTT with paracetamol and started corticoids treatment. The patient completed a two-week course of systemic corticosteroids with good clinical evolution, restoring renal function. Briefly, the proliferation of lymphocytes from the allergic patient was measured as previously described (6). Fresh peripheral-blood mononuclear cells separated over a density gradient (Histopaque-1,077, SigmaAldrich) and were incubated for 6 days at 106 cells/mL in triplicate with paracetamol (200, 100, 10, 1 µg/mL). Phytohemagglutinin (5 µg/mL) was the positive control. Proliferation was determined by the addition of [3 H] thymidine (0.5 µCi/well) for the final 18 hours of the incubation period. Proliferative response was calculated as stimulation index (SI), defined as the ratio between the mean values of counts per minute in cultures with antigen and those obtained without antigen. A positive response, defined as an SI over 2, was obtained with the drug tested. In summary, the result of the LTT with paracetamol was positive with a stimulation index of 4.1, 2.4, 0.7 and 2.0 for 1, 10, 100 and 200 µg/mL, respectively. The LTT has been used previously to assess delayed allergic reactions and due to its safety on the assessment of a T-cell response to the drug, it can be used to evaluate this type of reactions. The chronology of symptoms in our patient, its resolution following the withdrawal of the drug, and corticosteroid therapy, in addition to the complementary tests, suggests the diagnosis of paracetamol-induced acute tubulointerstitial nephritis, this being the first case reported that had been confirmed with LTT.

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Contributions

KP, MT-P: conceptualization, data collection, formal analysis. KP: writing – original draft. MT-P: writing – review & editing.

Conflict of interests

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