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3/2022

Rare anaphylaxis

Mothers' anxiety in infants food allergy

Specific IgE to a comprehensive panel of HDM allergens

Probiotic as an adjuvant therapy in chronic urticaria

Allergen sensitization associates with worse lung function parameters

CVID in ITP patients

Quality of life in severe asthmatic patients treated with benralizumab

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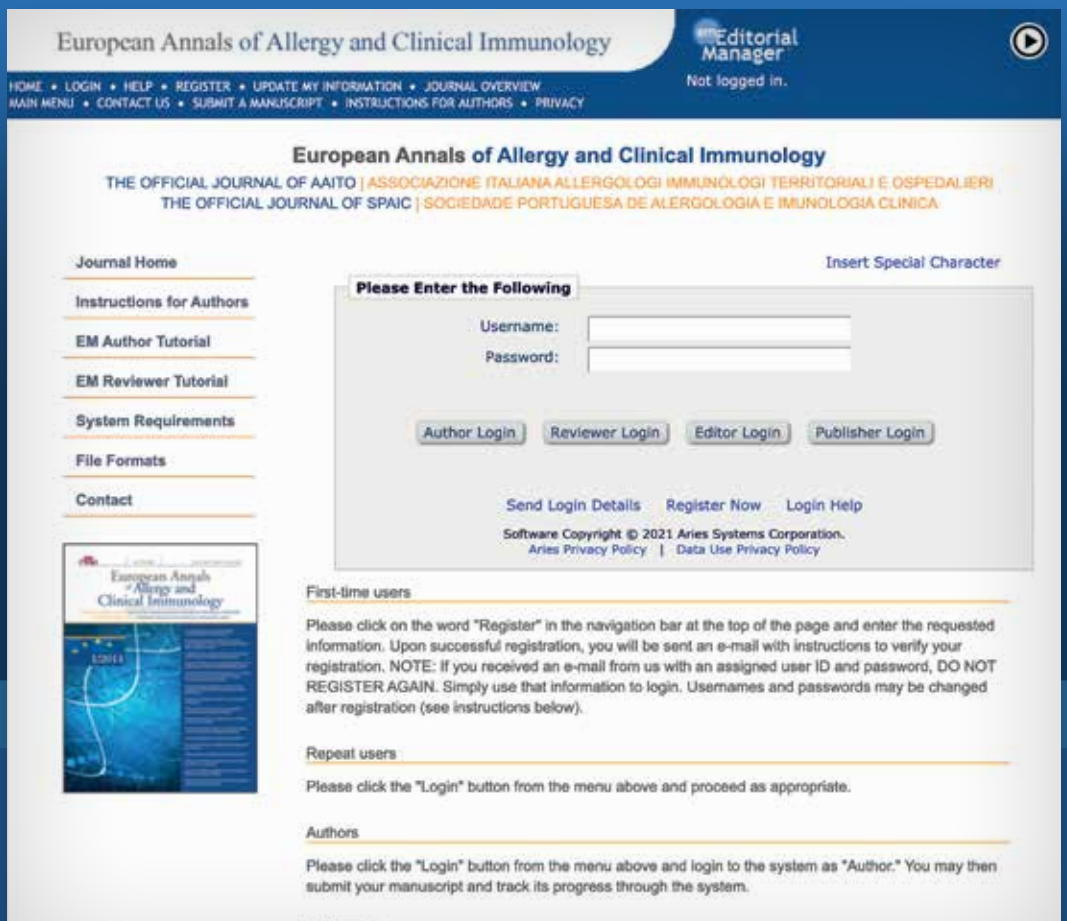

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Lessons from peculiar cases of anaphylaxis: why allergists should be prepared for the unexpected

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Adverse drug reaction; allergen exposure; anaphylaxis; bronchial asthma; Can f 5; COVID-19 vaccines; dog allergen; hypersensitivity; intimate behaviour; oxytocine; polyethylene glycol (PEG); psychological stress.

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Summary

Anaphylaxis is the most severe systemic hypersensitivity reaction, it can be caused by a number of well identified triggers such as foods, drugs, stinging insects and facilitated by predisposing clinical conditions. However, sometimes anaphylaxis shows up with uncommon or peculiar characteristics which could delay diagnosis and therapeutic treatment. In this report we aimed to describe less accounted/difficult-to-approach shapes of anaphylaxis to facilitate clinicians to suspect these severe reactions even in uncommon conditions. We choose to present data on anaphylaxis regarding simulation, mode of exposure to sensitizing agents, pregnancy, exposure to animals, intimate behaviour, psychological stress, and other situations.

Impact statement

Sometimes anaphylaxis can occur with uncommon or peculiar characteristics which could delay diagnosis and therapeutic treatment. We aimed to describe less accounted/difficult-to-approach shapes of anaphylaxis to facilitate clinicians to suspect these severe reactions even in uncommon conditions.

Introduction

Anaphylaxis is the most severe systemic hypersensitivity reaction, it involves multiple organ systems and can be deadly (1, 2). Anaphylaxis can be caused by a number of well identified triggers such as foods, drugs, stinging insects and clinical conditions (1, 2). However, sometimes anaphylaxis shows up with uncommon or peculiar characteristics which could delay diagnosis and therapeutic treatment. In this brief report we aimed to introduce less accounted/difficult-to-approach shapes of anaphylaxis to facilitate clinicians to suspect these severe reactions even in uncommon conditions.

Simulated anaphylaxis

In rare occasions, it has been reported that anaphylaxis can be mimicked by other clinic conditions (3) such as a diverticular perforation associated with colo-urachal fistula (4) and a massive subcutaneous emphysema presenting as anaphylaxis (5). We have recently shown that in obstetric surgery oxytocin/vasopressin can induce some side effects (negative inotropic and chronotropic effects, low blood pressure, vasodilatation) which can simulate a cardiac anaphylaxis and consequent intraoperative diagnostic problems and risks for the patient (6).

Anaphylaxis and mode of exposure

Inhalation mode

Inhalation of the offending allergens is an uncommon cause of anaphylaxis. Inhalant allergens such as those of some animals (guinea pig, horse, deer and rabbit) (7, 8) and grass pollens (9) can induce anaphylaxis. Food allergens, particularly from milk, in aerosolized form has been associated to anaphylaxis in a dairy worker (10) or in children as component of a medication aerosol (11). Anaphylaxis after occupational inhalation of Cefuroxime in a nurse has been also described (12).

Contact mode

It has been published only five cases of anaphylaxis induced by skin contact with milk or milk-derived foods; some indications on the risk factors to identify susceptible individuals are shown in **table I** (13). Recently a fatal reaction has been registered in a child at school (14) and another recent case report showed a generalized reaction induced by a cutaneous exposure to milk proteins in a colostrum-based cream (15).

Skin prick test (SPT)-related anaphylactic reactions can also be ascribed to the contact mode of exposure, those induced by the use of foods or drugs being the most frequent in clinical practice (16). On the contrary anaphylaxis produced by SPT with standard aero-allergens is extremely uncommon. We described a rare case of systemic life-threatening reaction after a routine SPT in a patient with high allergic sensitization to parietaria and grasses (17). In the same article we reported a multicentric evaluation of the frequency of SPT-related anaphylaxis in Italy: our case was the only one among 55.105 patients and 684.306 allergens tested (17). Possible risk factors for SPT-related anaphylaxis are similar to those described in **table I**. Moreover, in children we suggest to avoid the contemporary use of inhalant and food allergens, and the use of intradermal skin testing, to minimize the number of allergens to be tested during the SPT procedure, and to check the subjects for at least 20 min after the end of SPT. In some cases, it should be useful to consider the possibility of diluting allergenic extracts before their use (18, 19). It is important to underline that the need to carry out *in vivo* tests (*e.g.,* SPTs, prick by prick) for the diagnosis of food allergy in highly sensitized individuals has been reduced by the growing use and utility of modern laboratory procedures such as Component Resolved Diagnostics (20, 21).

Table I *- Possible predictive risk factors to identify patients with hazard for severe food allergic reactions through noningestant routes of exposure.*

High serum total IgE level
Strong family history of atopy
Early age of onset of symptoms despite having been breast-fed
Strong reactivity to skin-prick-tests or serum specific IgE evaluation
Clinical history
A disrupted skin barrier (*e.g.,* from atopic dermatitis, injury)

Modified from Liccardi G, De Falco F, Gilder JA, D'Amato M, D'Amato G. Severe systemic allergic reaction induced by accidental skin contact with cow milk in a 16-year-old boy. A case report. J Investig Allergol Clin Immunol. 2004;14(2):168-71.

Anaphylaxis and pregnancy

Anaphylaxis is considered uncommon during pregnancy. However, among potential risk factors such as age and history of allergy, caesarean delivery could be recognized as the main culprit condition because the necessity of using anaesthesia, surgical procedures and drugs (22). An amoxicillin-induced anaphylaxis at the end of pregnancy determined newborn death by hypoxic-ischaemic cerebral injuries (23).

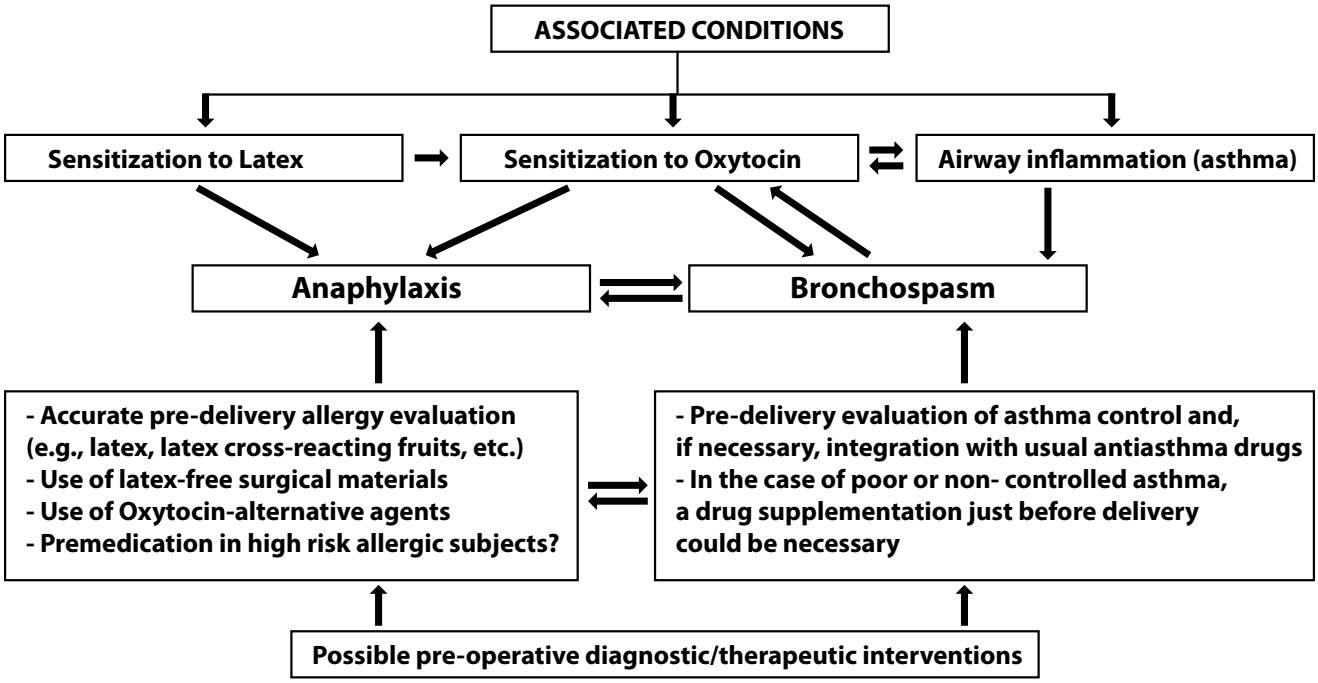
As previously reported, delivery, especially when carried out by caesarean section, can determine dangerous systemic reactions. Oxytocin constitute a complex cause of systemic/bronchial reactions as well as equivocal side effects-related- symptoms (negative inotropic and chronotropic effects, low blood pressure, vasodilatation) that mimic cardiac anaphylaxis (6, 24). Another insidious effect derived by a demonstrated homology in the protein sequence of oxytocin and latex allergens Hev b 7.01 and Hev b 7.02 (25).

We have previously described two life-threatening anaphylactic reactions with onset a few minutes after the infusion of oxytocin in two women sensitized to latex allergens during caesarean section under spinal anaesthesia in the delivery room (26). Moreover, it has been also demonstrated that oxytocin, under pro-inflammatory cytokines stimulation, may induce contraction of smooth muscle and airway narrowing suggesting that oxytocin serves as a bronchoconstrictor (27). These data suggest that inflammatory conditions of airways such as those found in asthmatic women (especially those with severe asthma) might constitute an independent (from anaphylaxis) risk factor for airway obstruction after infusion of oxytocin during delivery. The role of oxytocin receptors could also explain the well-known worsening of asthma control in about one-third of pregnant women suffering from asthma. These findings suggest a particular attention in managing delivering women suffering from latex allergy and bronchial asthma. An accurate anamnestic, clinical and diagnostic evaluation, a latex-free setting, the use of oxytocin-alternative agents and, in case of asthma, a drug premedication are likely to reduce the risk of anaphylactic or airway-obstructive reactions in these women (24) (**figure 1**).
Another extremely uncommon condition for anaphylaxis is lactation (28). It is not clear the pathogenesis, a possible role of hormones has been suggested (the withdrawal of the stabilising effect of progesterone on mast cells) but also the influence of others (prolactin, oxytocin, adrenocorticotropic hormone and corticotropin-releasing hormone) (29).
Finally, some case reports have shown that bovine serum albumin may be a causative agent in severe anaphylaxis after standard intrauterine insemination or *in vitro* fertilization if added to the culture medium of spermatozoids (30).

Anaphylaxis and animal exposure

Although respiratory allergy is a common consequence of animal allergens inhalation, in rare occasions a clear clinical picture of anaphylaxis developed after inhalation of guinea pig, horse, rabbit and deer epithelia (7, 8). Another possible modality of inducing anaphylaxis after exposure to animals is their bite. In some rare cases the bites were inflicted by domestic or wild animals such as horse, gerbil, mouse (31-33). However, the risk of anaphylaxis from animal bites is relatively more common in individuals working with laboratory animals, particularly rodents (34).
Another topic related to animals regards some food-derived animal substances that can be used in medications at various stages of the

Figure 1 *- Possible correlation between latex, oxytocin sensitization and airway inflammatory conditions including suggested pre-operative diagnostic/therapeutic interventions.*



Modified from Liccardi G, Bilò MB, Mauro C, Salzillo A, Piccolo A, D'Amato M, Liccardi A, D'Amato G. Oxytocin: an unexpected risk for cardiologic and broncho-obstructive effects, and allergic reactions in susceptible delivering women. Multidiscip Respir Med 2013;8(1):67.

manufacturing process. Caglayan-Sozmen *et al.* (35) review the possible roles of medications which may contain egg, red meat, gelatin, and fish allergens in allergic reactions in children with food allergy. The risk of reactions to hidden milk allergens in drugs is particularly important. In fact, dry-powder inhalers may contain lactose as excipient, this product can be rarely contaminated with milk proteins and, consequently, it may induce allergic reactions in patients with cow's milk allergy (36, 37). Reports have described immediate hypersensitivity reactions to methylprednisolone sodium succinate 40 mg injection, a formulation that contains lactose as excipient (38). Some cases of anaphylaxis after receiving diphteria-tetanus-pertussis vaccine injection (39) or some probiotics (40) in children allergic to milk have been reported. Among food-derived animal substances, also gelatin can induce systemic reactions being contained in medications like plasma volume expanders, erythropoietin, hemostatic products (41), as well as in some vaccines (42, 43). Although dogs can induce respiratory allergy, we would like to cite the potential role of service dogs in certain clinical conditions at high risk of anaphylaxis such as surgical interventions in subjects with high allergic risk. Tew and Taicher documented the first report of a service dog used to detect mast cell mediator release in patients with mastocytosis (44). This service dog was used not only in a family-centered care model, but also as an additional perioperative monitor to predict the eventual occurrence of perioperative mediator release (44).

Anaphylaxis and intimate behaviour

Although rare, the contact with allergens can also be due to a direct contact between two individuals, one of whom carries on his/her body the allergen potentially dangerous for the other individual (or can carry traces of food or drugs in biological fluids) (**figure 2**). This can easily happen during intimate behaviours, such as kisses or sexual intercourses. This topic may be underestimated and under-reported, due to its delicate nature, involving a very intimate aspect of the life (45).

The mechanism by which kissing may induce local or, in some cases, generalized reactions in sensitized individuals is the passive transport of allergenic molecules through saliva, skin or oral mucosa, and the consequent contact of these allergens with the skin or mucosae of the sensitized subjects. The severity of allergic symptoms likely depends on the type of kissing, as the contact with the 'unprotected' oral mucosa may account for a greater penetration of allergens in comparison to intact skin (45).

Foods are the most frequent cause of allergic reactions following a kiss from an individual who have eaten an allergenic food to a patient sensitized to the same allergen (46). Peanuts, walnuts and other tree nuts are the most common foods involved, followed by fruits or vegetables such as apple, carrot, kiwi, fish, shellfish, and milk (47, 48). There are only few reports of oral allergy syndrome and consequent generalized urticaria in drug-sensitized individuals, a few minutes after passionate kisses given by their partners who had previously used the drug particularly beta lactams (49, 50). Foods (such as Brazil nut) can also penetrate into seminal fluid and induce after vaginal intercourse (51); seminal fluid can also carry antibiotics (such as amoxicillin) and trigger anaphylaxis in an amoxicillin-sensitized woman after oral sex (52). Anaphylaxis after anal intercourse but tolerance after vaginal intercourse has been also described (53).

Seminal plasma hypersensitivity (SPH) is characterized by immediate local or systemic postcoital symptoms following mucosal exposure to seminal fluid. Systemic SPH is a type I, immediate, IgE-mediated hypersensitivity reaction with the well-known symptoms (54).

The prostate-specific antigen (PSA), a 33-34 kDa glycoprotein, better known for its use in monitoring prostate cancer in men, was in fact identified as a major allergen in human seminal fluids. It was found that PSA carries high homology to the canine prostatic kallikrein, which was identified as Can f 5. The clinical relevance of this cross-reactivity was confirmed in a few cases, and investigators have hypothesized that patients who experienced SPH after their first unprotected intercourse could have been sensitized by previous exposure to dogs (54).

We have recently shown the increasing relevance of allergic sensitization to Can f 5 in North East Italy considering both the number of sensitized individuals (69.02% among 268 dog-sensitized ones, and 57.92% as exclusive sensitization) and the level of sensitization (77.6% of individuals showed medium or high values of specific IgE according to manufacturer's specifications) (55). Moreover, our Italian multicenter study has shown that a prevalent exposure to a male dog represents a risk factor for the presence of Can f 5 monosensitization (56). The results of this study emphasize the need of an adequate diagnosis and management of patients suffering from dog allergy, especially those with relevant clinical symptoms following dog exposure in order to prevent systemic SPH (57).

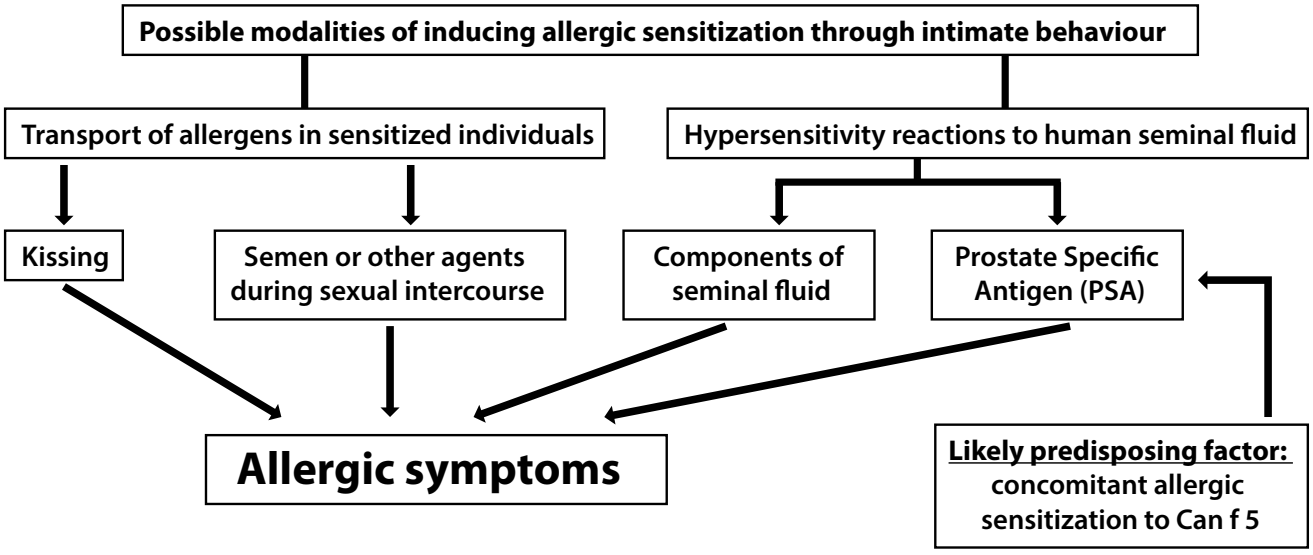
Anaphylaxis and psychological stress

The relationship between anaphylaxis and psychological stress (PS) is an intriguing puzzle, the question being: can the former be the cause or only consequence of the latter or both?

It is well documented that (PS) may enhance allergic diseases such as asthma, allergic rhinitis, and atopic dermatitis through several complex mechanisms like mast cell activation and mediator release, inflammation and imbalance of the autonomic system (58-60).

Stress can activate the hypothalamic-pituitary-adrenal axis through the release of corticotropin-releasing hormone (CRH). Theoharides *et al.* have shown that CRH secreted under stress stimulates mast cell degranulation through activation of CRH receptor-1 (CRHR-1) (61). This effect is augmented by other neuropeptides also released by stress, such as substance P (SP) and neurotensin (NT). In fact, both NT and SP induce the expression of functional

Figure 2 *- Possible modalities of inducing allergic sensitization through intimate behaviour.*



Modified from Liccardi G, Caminati M, Senna G, Calzetta L, Rogliani P. Anaphylaxis and intimate behaviour. Curr Opin Allergy Clin Immunol 2017;17(5):350-5.

CRHR-1 (61). Moreover, the same authors have shown that CRH induces the expression of high-affinity IgE receptor (FcεRI) and augments allergic stimulation of human mast cells (62).

To the best of our knowledge, only two case reports have clearly suggested a relationship between a PS situation and the onset of anaphylaxis (63, 64). The diagnosis of such anaphylaxis has been made essentially through anamnestic criteria and exclusion of other most common triggering agents/situations. We believe that PS alone or as relevant co-factor could induce anaphylaxis more frequently than expected if we consider the aforementioned mechanisms and the high presence of PS in the general population. On the contrary, the role/onset of PS in patients surviving anaphylaxis episodes has been better explored. Baiardini *et al.* (65) studied quality of life and well-being in patients with drug-induced anaphylaxis. Two validated tools were used: The Drug Hypersensitivity Quality-of-Life Questionnaire (DrHy-Q) and the Psychological General Well-Being Index (PGWBI). Compared with the Italian reference population, patients had a significantly reduced PGWBI total and domain score. The authors highlighted for the first time impaired Health Related Quality of Life (HRQoL) and distress commonly feature in survivors to anaphylactic reactions to drug.

Recently Lee *et al.* (66) have evaluated Posttraumatic Stress Disorder (PTSD) arising after anaphylaxis in adults, by investigating the psychosocial burden of recent anaphylaxis in Korean adults. The results of this study indicated that patients who experienced anaphylaxis were likely to develop psychiatric disorders, such as PTSD, anxiety, and depression. The development of PTSD did not appear to depend on the severity of anaphylaxis, although patients with higher Impact of Event Scale-Revised-Korean version (IES-R-K) scores had more severe anxiety and depression. These and previous considerations suggest that the general management (diagnosis/treatment) of anaphylaxis should include a psychological/psychiatric evaluation.

Others

Anaphylaxis after the use of polyethylene glycol (PEG), a water-soluble, organic compound included in a wide variety of products has been described. In the healthcare setting, it is a common ingredient in medications and procedural agents (*e.g.,* for performing ultrasound scans) (67). Anaphylaxis to chlorhexidine (a synthetic bisbiguanide antiseptic agent) has been reported particularly in the perioperative and medical procedural settings (68).

Rare cases of anaphylaxis have been reported also with the use of anti-anaphylaxis agents such as cetirizine and chlorpheniramine (69, 70). Another controversial topic is the possible induction of anaphylaxis by vaccines used to prevent contagious infections, many patients refusing vaccinations for this fear. Mc Neil *et al.* (71) reported only 33 vaccine-triggered anaphylaxis cases that occurred after 25,173,965 vaccine doses (against different infectious agents), the rate of anaphylaxis being 1.31 (95% CI, 0.90-1.84) per million vaccine doses.

Interest in this issue has increased significantly with the recent start of mass vaccination against the Sars-CoV-2 virus. Preliminary reports have been published on anaphylactic events occurring after the administration of the first Sars-CoV-2 available vaccines. Twenty-one cases were determined to be anaphylaxis (a rate of 11.1 per million doses administered) after the use of Pfizer-BioNTech COVID-19 Vaccine (72), while 10 cases were determined to be anaphylaxis (a rate of 2.5 anaphylaxis cases per million Moderna COVID-19 vaccine doses administered) (73). The aforementioned polyethylene glycol (PEG) has been indicated as potential agent of anaphylaxis in these vaccines being an excipient (74-76).

Conclusions

Data reported from our previous studies and from a review of the Literature show that sometimes anaphylaxis can occur with uncommon or peculiar characteristics which could delay diagnosis and therapeutic treatment. We hope that the description of less accounted/difficult-to-approach shapes of anaphylaxis could facilitate clinicians to suspect these severe reactions even in less common clinic presentations.

Previous presentations

Data presented at AAIITO 2020. Online "Il Mese dell'Allergologia AAIITO" 9-26 October 2020.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Effects of elimination diets and clinical findings on mothers' anxiety in infants with food allergy with non-life-threatening reactions

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Key words

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Impact statement

The anxiety of the mothers of the infants with food allergy were higher than the control group, and the state anxiety scores higher than the mothers of infants with food protein-induced allergic proctocolitis and multiple food elimination.

Summary

Background. *In food allergies (FA), the current treatment is eliminating the responsible food from the diet until tolerance develops. We aimed to determine the effects of the mother's elimination diets and clinical findings on the mothers' anxiety in infants with food allergy (FA) with non-life-threatening reactions.* ***Methods.*** *Our study included 100 infants with FA and 35 healthy infants as control. A data form was prepared to collect information about the infants' and their mothers' socio-demographic characteristics, clinical findings, features of the mother's elimination diet, and FA-related internet search. State-Trait Anxiety Inventory (STAI) was applied to all mothers.* ***Results.*** *The STAI (state anxiety and trait anxiety) scores of the mothers of the infants with FA were higher than the control group ($p < 0.001$, $p = 0.001$, respectively). Of the infants with FA, 51% had food protein-induced allergic proctocolitis (FPIAP), 29% had atopic dermatitis (AD), 20% had urticaria-angioedema (U/AE). It was found that state anxiety scores were higher in mothers whose child had FPIAP, who had multiple food eliminations, who followed the allergy groups on social media, and who made the elimination diet herself ($p = 0.008$, $p = 0.048$, $p < 0.001$, $p = 0.001$, respectively).* ***Conclusions.*** *The state anxiety and trait anxiety of the mothers of the infants with FA were higher than the control group, and the state anxiety scores were higher especially in the mothers of infants with FPIAP and multiple food elimination.*

Introduction

Food allergies (FAs) have becoming increasingly more common throughout the world in recent years. Recent studies have shown that the prevalence of FA has increased by up to 10%, mainly in children (1-3). Cow's milk allergy (CMA) and egg allergy are the most common food allergies in infants (4, 5). There is no curative treatment that has been put into routine use yet despite the attempts for oral immunotherapy in peanut, milk, and egg allergies (6).

The current approach for management infants with FAs is to eliminate the offending food that causes allergic reactions until they develop a tolerance, 1) to educate the family about emergency situations and their management in case of accidental intake, 2) to follow up the growth and development of the infant, and 3) to follow-up the development of tolerance. It has been shown that in severe cases of FAs, the risk of life-threatening reactions after accidental intake increases the anxiety of families and children and, therefore, may lead to severe restrictions in their social lives and a decrease in quality of life (7-10). Similarly, studies have shown that mothers of children with food allergy with non-life-threatening reactions have high anxiety, impaired quality of life, and even unnecessarily restricted elimination diets (11, 12). However, in children with non-life-threat-

ening reactions, the mothers' anxiety level and the affecting factors are not clearly known. The state-trait anxiety inventory (STAI) is a well-standardized, 40 item questionnaire designed as a self-report instrument to evaluate both state and trait anxiety. The STAI is a tool widely used for the screening of anxiety in the population (13). In this study, we aimed to investigate the elimination diet practices and anxiety levels of mothers of infants with different clinical findings and with FA with non-life-threatening reactions.

Materials and methods

Study design

This cross-sectional survey study was conducted between September 2018 and September 2019 in a tertiary referral Pediatric Allergy and Immunology with Child Health and Psychiatry outpatient clinics.

Ethics and consent

Our university Institutional Review Board approved this study (Approval #: KA18/251). Verbal and written consent of the parents of the children with FA were obtained before participating in the study. All study procedures were conducted following the Declaration of Helsinki and local laws and regulations.

Participants

Infants aged 0-1 year who were referred to our pediatric allergy outpatient clinic with suspicion of FA and diagnosed with CMA and/or egg allergy were included in the study. Healthy children followed for growth development within the same age group constituted the control group. The patients were assigned into three groups based on the clinical finding as follows: food protein-induced allergic proctocolitis (FPIAP), atopic dermatitis (AD), urticaria-angioedema (U/AE). Infants with food allergies other than cow's milk and/or egg, infants with FA with life-threatening reactions (such as anaphylaxis and food protein-induced enterocolitis), and infants with chronic diseases were excluded from the study. In addition, infants with severe atopic dermatitis and infants with exacerbation of atopic dermatitis in the last month were also excluded from the study. Mothers with chronic diseases that may affect their psychological status and mothers with psychiatric diseases with a doctor diagnosis were excluded from the study.

Evaluation of FA

All patients were subjected to a detailed evaluation of the history of FA. The diagnosis of FA was based on the criteria suggested by the European Academy of Allergy and Clinical Immunology (EAACI) in the food allergy and anaphylaxis guidelines (1). The diagnosis of FPIAP was based on the same guidelines (history, improvement of symptoms by eliminating the offending food, recurrence of symptoms following oral food challenge-OFC). OFC was performed to mothers and/or children after 2-4 weeks of eliminating the suspected foods. The AD diagnosis was based on the Hanifin-Rajka criteria (14). All patients with AD and U/AE underwent skin prick-test (SPT) with food allergens (cow's milk, egg, wheat flour, soy flour, tree nuts, sesame, peanut, fish) by using an allergen test solution (ALK, Denmark) and prick-to-prick test (cow's milk and egg). OFC was performed in all children after 2-4 weeks of eliminating the suspected foods (1). The children were followed up every three months until tolerance developed.

Data collection

Socio-demographic data form

After evaluation of FA, the mothers of infants have filled out a socio-demographic data form. This form was prepared to collect data about the socio-demographic characteristics of the mothers and the infants, such as age, sex, maternal education and employment, the number of siblings, type of delivery, whether the mother received support for the infant's care. Also, in this form, the time of onset of clinical findings, the person who made the elimination diet (mother, infant, mother and infant together), the number of avoided food, the person who recommends starting the elimination diet (self-decision, doctor's recommendation, friend's recommendation, internet research), the time spent on the FA-related internet search and whether the mother followed an allergy group on social media were questioned.
The children were categorized according to the number of foods that they eliminated. If they eliminated three or more foods, they were categorized in the multiple food elimination group.

State-Trait Anxiety Inventory (STAI)

The State-Trait Anxiety Inventory (STAI) is a self-reported questionnaire composed of 40 items developed to evaluate two different types of anxiety: state anxiety (emotional condition transitory), whose reference frame is the "now, at this moment", and the anxiety trait (anxiety tendency relatively stable), whose reference frame is "in general, in most of the times". The STAI has a Likert-type response format with four options (1 = almost never/nothing; 2 = some/sometimes; 3 = quite/often; 4 = a lot/almost always). Each graded from a minimum score of 20 to a maximum of 80, which indicates greater anxiety (13). A cut-off score of 40 is commonly used to define probable clinical levels of anxiety (15). A Turkish version of Spielberger's STAI was validated and previously used to measure mothers' anxiety levels (16). The internal reliability of the Turkish version was 0.94-0.96 for state anxiety and 0.83-0.87 for trait anxiety.

Statistical analysis

G*Power 3.1.9.2 (Düsseldorf University, Germany) program was used to calculate the sample size of the study. It was calculated that the total sample size should be at least 81, given the

margin of error (α-error) was 0.05, the effect size was 0.4, and the power of the test (1-β error) was 80% (goodness-of-fit tests for contingency tables) (17).

Statistical analyses were performed using IBM SPSS 21.0 (Statistical Package for Social Sciences, SPSS, Inc., Chicago, IL). The results were expressed as the number of cases (percentage) for categorical data or mean ± standard deviation for continuous data. Non-normally distributed data were presented using medians, minimum-maximum, and interquartile range (IQR). Non-normally distributed data were compared by Kruskal-Wallis tests. Non-parametric tests (the Mann-Whitney U test or the Kruskal-Wallis test) were used to compare data. Spearman's rho test was used for the correlation analysis. For the multivariate analyses were further entered into the logistic regression analysis to determine independent predictors of patient outcome. Hosmer-Lemeshow goodness of fit statistics were used to assess model fit. P-values < 0.05 were considered statistically significant.

Results

The study included a total of 100 children with FAs and 35 healthy children. The median age of children with FAs was 4.0 (3.0) [1-12] months, and 58% were females. The median age of the control group was 5.0 (3.0) [1-8] months, and 42.9% were females. No significant difference was found between children with and without FAs in terms of age, gender, maternal age, education status, employment, the number of siblings, type of delivery, whether the mother received support for the infant's care. The mothers of children with FAs had significantly higher state anxiety ($p < 0.001$) and trait anxiety ($p = 0.001$) scores compared to those in the control group. The demographic data of the children and their mothers' and anxiety scores were given in **table I**.

Of those with FAs, 51% had FPIAP, 29% had AD, and 20% had U/AE. All of the cases were on an elimination diet for at least one food before evaluation of FA. The rate of initiating an elimination diet with mothers' self-decision was 33.4% in the FPIAP group, 20.7% in the AD group, and 15% in the U/AE group. The most common dietary regimen was multiple food elimination. The highest rate of multiple food elimination was in the FPIAP group (74.5%), with a significant difference ($p = 0.008$). The most significant reduction in the number of eliminated foods after the evaluation of FA was in the FPIAP group (76.5%) ($p < 0.001$). The comparison of groups regard-

Table I *- Demographical data of patients and healthy controls, expressed as n (%).*

	Patients (n = 100)	**Control (n = 35)**	**P-value**
Age of children, months*	4.0 (3.0) [1-12]	5.0 (3.0) [1-8]	0.668
Female	58 (58.0)	15 (42.9)	0.122
Age of mothers, years†	31.4 ± 4.7	30.5 ± 3.3	0.276
Maternal education level			
High school	27 (27.0)	10 (28.6)	0.858
University	73 (73.0)	25 (71.4)	
Maternal employment	42 (42.0)	16 (45.7)	0.702
Number of children in the family			
1	59 (59.0)	19 (54.3)	0.627
≥ 2	41 (41.0)	16 (45.7)	
Cesarean section (C/S)	73 (73.0)	30 (85.7)	0.128
Severe infantile colic	51 (51.0)	11 (31.4)	**0.046**
Assistance on child care (Grandparents/nanny)	34 (34.0)	14 (40.0)	0.523
Paternal assistance on child care	65 (65.0)	28 (80.0)	0.099
State anxiety scores†	44.8 ± 11.7	37.0 ± 5.8	**< 0.001**
Trait anxiety scores†	42.7 ± 9.2	37.8 ± 6.8	**0.001**
State anxiety scores ≥ 40	61 (61)	14 (40.0)	**0.031**
Trate anxiety scores ≥ 40	63 (63)	12 (34.3)	**0.003**

*Data expressed as median (inter quartil range) [minimum-maximum]; †data expressed as mean ± SD.

ing their demographic data, features of the elimination diet were shown in **table II**.

The relationship between mothers' anxiety levels and the factors that may affect anxiety levels were shown in **table III**. The state anxiety scores were significantly higher in mothers who had an infant with FPIAP (compared to those with U/AE, p < 0.001), in mothers who were on the elimination diet alone (compared to those on the elimination diet together with the infant, p = 0.001) and who had multiple food eliminations (p = 0.048) (**figure 1**).

State and trait anxiety scores were found to be higher in mothers who followed allergy groups on social media (p < 0.001, p = 0.002, respectively). State anxiety scores were found to be higher in mothers who spent more than 1 hour on the FA-related internet search

Table II *- Comparison of patients according to diagnosis, expressed as n (%).*

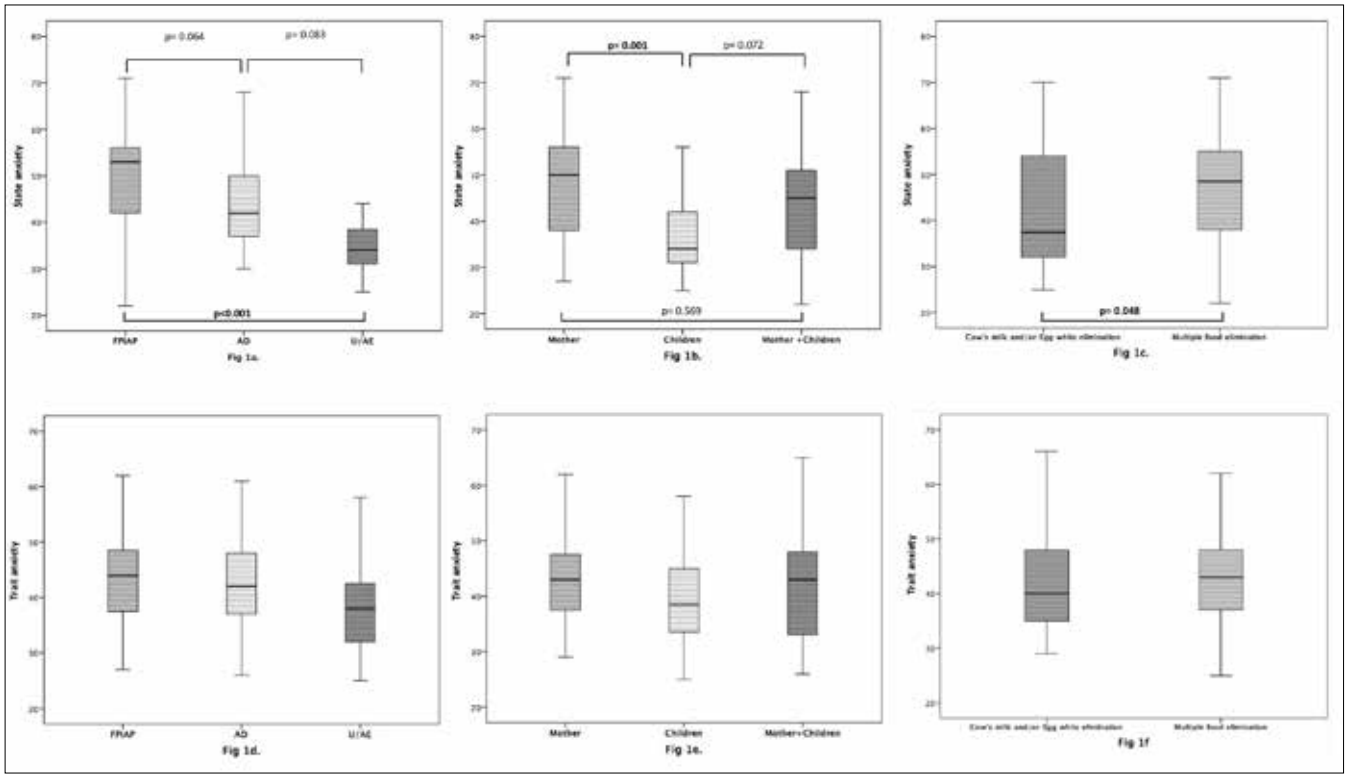
	FPIAP (n = 51)	AD (n = 29)	U/AE (n = 20)	P-value
Age of children, months*	3.0 (1.0) [1-5]	4.0 (5.0) [1.5-12]	6.0 (5.0) [2-12]	0.039
Female	31 (60.8)	16 (55.2)	11 (55.0)	0.847
Cesarean section (C/S)	39 (76.5)	19 (65.5)	15 (75.0)	0.555
Severe infantile colic	34 (66.7)	10 (34.5)	7 (35.0)	0.006
Self-reported anxiety during pregnancy	19 (37.3)	15 (51.7)	10 (50.0)	0.380
Self-reported anxiety during breastfeeding	38 (74.5)	18 (62.1)	9 (45.0)	0.059
Assistance on child care (grandparents/nanny)	16 (31.4)	11 (37.9)	7 (35.0)	0.833
Paternal assistance on child care	37 (72.5)	15 (51.7)	13 (65.0)	0.172
Food allergy releated internet search				
< 1 hour	1 (2.0)	4 (13.8)	5 (25.0)	0.010
≥ 1 hours	50 (98.0)	25 (86.2)	15 (75.0)	
Following a family support group on social media	39 (76.5)	19 (65.5)	4 (20.0)	< 0.001
Age at the onset of symptoms (mo)†	1.7 ± 0.7 (1.0-3.0)	2.4 ± 1.9 (1.0-12.0)	4.2 ± 1.9 (1.0-6.0)	0.596
Person on the diet				
Mother	37 (72.5)	10 (34.5)	0 (0.0)	< 0.001
Children	0	3 (10.3)	17 (85.0)	
Both	14 (27.5)	16 (55.2)	3 (15.0)	
Initial suggestion of diet				
Physician	34 (66.6)	23 (79.3)	17 (85.0)	0.068
Internet search	16 (31.4)	4 (13.8)	1 (5.0)	
Family member/friend	1 (2.0)	2 (6.9)	2 (10.0)	
Eliminated food(s) before evaluation of FA				
Cow's milk and/or egg	13 (25.5)	12 (41.4)	13 (65.0)	0.008
Multiple food elimination (≥ 3 foods)	38 (74.5)	17 (58.6)	7 (35.0)	
No. of eliminated foods before evaluation of FA				
1	9 (17.6)	1 (3.4)	10 (50.0)	< 0.001
2	4 (7.8)	11 (37.9)	3 (15.0)	
≥ 3	38 (74.5)	17 (58.6)	7 (35.0)	
Eliminated foods after evaluation of FA				
Cow's milk and/or egg	49 (96.1)	22 (75.9)	16 (80.0)	0.021
Multiple food elimination (≥3 foods)	2 (3.9)	7 (24.1)	4 (20.0)	
Number of eliminatied food after evaluation of FA				
1	38 (74.5)	11 (37.9)	11 (55.0)	0.012
2	11 (21.6)	11 (37.9)	5 (25.0)	
≥ 3	2 (3.9)	7 (24.1)	4 (20.0)	
Decrease in no. of foods eliminated	39 (76.5)	17 (58.6)	5 (25.0)	< 0.001

*Data expressed as median (inter quartil range) [minimum-maximum]; †data expressed as mean ± SD.

Table III *- Comparison of anxiety levels in mothers of patients, expressed as mean ± SD (min-max).*

	State anxiety	P-value	Trait anxiety	P-value
Diagnosis				
FPIAP	49.3 ± 11.7 (22-71)	**0.008**	44.1 ± 8.5 (27-62)	0.471
AD	43.1 ± 9.7 (30-68)		43.1 ± 10.6 (26-66)	
U/AE	35.8 ± 8.0 (25-63)		38.7 ± 8.4 (25-58)	
Severe infantile colic				
No	41.2 ± 11.0 (22-70)	**0.002**	42.3 ± 10.1 (25-66)	0.567
Yes	48.3 ± 11.3 (30-71)		43.1 ± 8.4 (29-62)	
Maternal education level				
Compulsory education/High school	42.8 ± 9.8 (29-58)	0.260	41.3 ± 9.3 (26-66)	0.258
University	45.6 ± 12.2 (22-71)		43.2 ± 9.2 (25-65)	
Maternal employment				
No	43.6 ± 11.0 (22-71)	0.282	41.8 ± 9.3 (25-66)	0.198
Yes	46.6 ± 12.4 (30-70)		44.0 ± 9.1 (25-65)	
Number of children in the family				
1	44.7 ± 11.1 (27-71)	**0.016**	42.4 ± 7.9 (26-62)	0.130
≥ 2	40.1 ± 10.4 (22-68)		40.1 ± 10.4 (25-66)	
Self-reported anxiety during pregnancy				
No	43.2 ± 11.8 (22-70)	0.119	40.4 ± 8.2 (25-58)	**0.014**
Yes	46.9 ± 11.3 (31-71)		45.6 ± 9.7 (29-66)	
Self-reported anxiety during breastfeeding				
No	39.5 ± 11.1 (22-70)	**< 0.001**	39.1 ± 8.8 (25-59)	**0.004**
Yes	47.7 ± 11.0 (27-71)		44.7 ± 8.9 (29-66)	
Assistance on child care (grandparents/nanny)				
No	46.3 ± 12.5 (22-71)	0.103	43.5 ± 9.8 (25-66)	0.320
Yes	41.9 ± 9.3 (27-58)		41.2 ± 8.0 (26-60)	
Paternal assistance on child care				
No	43.1 ± 10.4 (27-68)	0.299	43.3 ± 9.7 (30-66)	0.862
Yes	45.8 ± 12.3 (22-71)		42.4 ± 9.1 (25-62)	
Person on the diet				
Mother	48.0 ± 11.3 (27-71)	**0.002**	43.7 ± 8.2 (29-62)	0.304
Children	37.3 ± 9.3 (25-63)		40.7 ± 10.5 (25-66)	
Both	44.8 ± 11.6 (22-68)		42.5 ± 9.8 (26-65)	
Initial suggestion of diet				
Physician	44.4 ± 11.6 (22-70)	**0.041**	42.5 ± 9.3 (25-66)	**0.032**
Internet/Social media	48.9 ± 11.4 (30-71)		45.2 ± 8.9 (26-61)	
Family member/friends	33.8 ± 4.7 (27-39)		35.0 ± 5.7 (30-42)	
No. of eliminated foods before evaluation of FA				
1	43.4 ± 13.8 (27-70)	0.150	42.0 ± 9.0 (30-59)	0.898
2	40.7 ± 10.6 (25-63)		43.3 ± 10.9 (29-66)	
≥ 3 foods	46.5 ± 11.0 (22-71)		42.8 ± 9.0 (25-65)	
Eliminated food(s) before evaluation of FA				
Cow'milk and/or Egg	42.1 ± 12.3 (25-70)	**0.048**	42.6 ± 9.8 (30-59)	0.853
Multiple food elimination (≥3 foods)	46.5 ± 11.0 (31-68)		42.8 ± 9.0 (25-65)	
Decrease in no. of foods eliminated after evaluation of FA				
No	46.2 ±11.4 (25-70)	0.136	42.9 ± 9.0 (25-66)	0.763
Yes	42.6 ± 11.8 (22-68)		42.4 ± 9.7 (25-63)	
Food allergy releated internet search				
< 1 hour	38.2 ± 9.6 (25-57)	0.068	40.5 ± 9.1 (30-62)	0.930
≥ 1 hours	45.6 ± 11.7 (22-70)		43.0 ± 9.3 (25-66)	
Following an allergy group on social media				
No	37.9 ± 9.9 (22-68)	**< 0.001**	39.2 ± 8.5 (25-60)	**0.002**
Yes	49.1 ± 10.6 (29-71)		44.9 ± 9.0 (26-66)	

Figure 1 *- Comparison of mothers' state and trait anxiety scores according to (**a, d**) clinical findings (p = 0.052), (**b, e**) who did the elimination diet (p = 0.304), and (**c, f**) characteristic of elimination diet (p = 0.853).*



but not statistically significant (p = 0.068). A positive correlation was found between the state and trait anxiety scores (r = 0.656 and p < 0.001). When the factors affecting state and trait anxiety scores were analyzed with logistic regression analysis, it was determined that following allergy groups on social media was effective in both state and trait anxiety scores (**table IV**) (p = 0.001, p = 0.003).

Discussion

In our study, it was determined that the anxiety levels of mothers with children with FA were higher than healthy children, and most of them had a restricted diet more than necessary before the allergy work-up. Among mothers, it was determined that elimination of multiple foods was high, especially in patients with FPIAP, and

Table IV *- Evaluation of state and trait anxiety scores of mothers whose children with asthma by logistic regression analysis.*

	State Anxiety Scores ≥ 40		Trait Anxiety Scores ≥ 40	
Risk Factor	**RR (95% CI)**	**P-value**	**RR (95% CI)**	**P-value**
Maternal Age	0.93 (0.83-1.04)	0.196	1.05 (0.95-1.16)	0.334
Highly educated mother	1.87 (0.57-6.15)	0.305	1.43 (0.48-4.26)	0.527
Working Mother	0.47 (0.16-1.37)	0.165	1.16 (0.42-3.22)	0.775
Number of children in the family (1 child)	1.69 (0.64-4.50)	0.294	0.45 (0.17-1.17)	0.101
No assistance on child care	1.55 (0.57-4.22)	0.387	0.95 (0.35-2.53)	0.914
Following an allergy group on social media	5.77 (2.08-15.97)	0.001	4.45 (1.67-11.82)	0.003
Multiple food elimination (≥ 3 foods)	2.00 (0.76-5.29)	0.160	0.81 (0.31-2.14)	0.673

about three-quarters of them had multiple food elimination either by the family's own decision or by the doctor's recommendation. After the allergy work-up, multiple food allergy was only 3.9% of children with FPIAP. In addition, it was found that the state anxiety levels were higher in mothers of children with FPIAP, mothers who eliminated multiple food, and mothers who followed allergy groups on social media compared to other groups.

In our study, all families of children had started an elimination diet before the evaluation of FA, and a third of these families decided to start the elimination diet by themselves. Studies have shown that 26-76% of the families of children with FAs had started the elimination diet themselves before the evaluation of FA, and even a restricted diet could be applied in children (8, 11, 18, 19). Beken *et al.* (11) found that 85.9% of the patients evaluated with food allergy suspicion applied food elimination before the evaluation of FA, and even 45.9% of these patients applied multiple food elimination. In this study, it was shown that only 40% of patients had food allergies after OFC. In our study, after the allergy work-up, three-quarters of children with FPIAP, half of children with AD, and one-fourth of children with U/AO had a decrease in the number of eliminated foods. Currently, the only established treatment method for FAs is to avoid suspicious foods, but an unnecessary stringent elimination diet may lead to nutritional deficiencies in the mothers and children (20).

Studies have found that children with life-threatening FA and their families have higher anxiety scores due to the risk of anaphylaxis after accidental food intake (21-23). It has been shown that mothers' anxiety levels are also high in non-life-threatening food allergies such as FPIAP and mild AD. It has been shown that the unproven diagnosis of food allergy and symptoms are effective on the mother's anxiety level, especially in late-type FA (11, 21). Cortes *et al.* (24) have also found that the mother's anxiety was related to symptoms, especially in children with gastric and cutaneous symptoms. Beken *et al.* (11) found that mothers of children with food allergy aged 0-2 years had higher levels of state and trait anxiety than the control group, and state anxiety levels decreased when food allergy was excluded after OFC in mothers who started the elimination diet themselves. In our study was found that the mothers who had infants with FAs had significantly higher state anxiety scores than the control group. In addition, state anxiety scores were higher in mothers who eliminated multiple food. Deschildre *et al.* (25) found that a restricted diet in children with a peanut allergy was associated with an increase in the family's anxiety level. It is not clear in the studies whether mothers with anxiety tend to have a more restricted diet or whether a restricted diet increases anxiety. In order to explain this situation, there is a need for more studies in which mothers' anxiety is monitored for a long time and evaluated repeatedly. Interestingly, in our study also was found that the mothers who had infants with FAs had significantly higher trait anxiety scores than the control group. This was an unexpected result for us. Similar to our study, Avcıl *et al.*'s study found that mothers of children with asthma had higher both stait and trait anxiety levels than the control group (26). Recent studies have suggested a close association between prenatal maternal distress and allergic diseases in the offspring. Prenatal maternal distress can lead to steroid imbalance and oxidative stress, which can be a risk factor for the development of allergic diseases in offspring (27). Kim *et al.*'s study, genome-wide DNA methylation microarray and pyrosequencing were used to prove epigenetic change for the association between maternal anxiety during pregnancy and AD development in the offspring. This result has been shown to be associated with DNA methylation of MMP27 (28). Although there are studies that support maternal anxiety as a risk for the development of allergic diseases in their children, this is not clear. Today, it is effortless to access information through internet searches, but information pollution and the inability to differentiate appropriate information sources may confuse families (29). Some studies have shown that supporting parents' access to educational materials about their children's disease reduce their anxiety and improve their quality of life (30). It has been a common trend that the families of children with chronic diseases follow blogs related to the subject on social media/internet or get involved in the disease-specific support groups. Although these platforms provide social support to families, they may sometimes affect the families negatively and produce the opposite outcome due to the access to information that will further increase their anxiety, excessive generalization, and taking wrong examples (31, 32). Beken *et al.* (11) found that FA-related internet research was more common in mothers who decided to start a food elimination themselves. In our study, detailed data on the content of the internet research could not be obtained and the data were recorded only according to the report of the mother. However, in our study, it was found that anxiety levels were higher in mothers who spend more time on the FA-related internet search and follow an allergy group on social media. We think that this result can be explained as mothers with high basal anxiety levels want to do more research, or mothers confuse because of the accuracy of the information they learn as a result of internet research or whether it is related to the disease of their child.

As a limitation of our study, the anxiety of the mothers was not re-evaluated. Since our study is a cross-sectional study, the anxiety could not be re-evaluated, but we think that re-evaluation of the anxiety may explain the effects of elimination diet on the psychological status of the mothers more clearly. Another limitation of our study was that patients without milk and or egg allergies were not evaluated. However, we evaluated these foods because they are the most frequently encountered allergies in practice, and the most commonly consumed foods in this age group. The heterogeneity of the clinical findings of the patients also seems to be a limitation of our study, but the patients with severe symptoms (anaphylaxis, severe eczema, *etc.*) were excluded from the study, considering that it may affect the anxiety of the mothers. This study was conducted in a private university

hospital, and the mothers' education levels were higher than the general population. Therefore, we think that it would be appropriate to compare this study with a multi-center study with a larger number of patients to represent the general population. Demonstrating that the clinical findings, characteristic of elimination diet, and the internet usage habits affect the mothers' anxiety level who have children with FAs was the strength of our study.

Conclusions

Understanding the concerns of the families of children with FA, educating them about what to do in an emergency, informing them about the natural course of the disease will help them control their anxiety and make it easier for families to cope with the disease. A multidisciplinary treatment approach, including dietary practices and psychological and social support, should be used for the families with children with FAs who were recommended an elimination diet.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Evaluation and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens using a new multiplex assay: a real-life experience on an Italian population

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Key words

House dust mites (HDM) allergy; specific IgE; allergens; component resolved diagnosis (CRD); Multiplex assay.

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Impact statement

Assessment to a comprehensive profile of HDM allergens defines serological rectivity profiles that seem associate with different clinical presentations.

Summary

Background. *House dust mites (HDM) are among the most important allergen sources worldwide, representing a major cause of perennial allergic rhinitis and asthma.* ***Aim.*** *To evaluate the prevalence of IgE responses towards a comprehensive panel of HDM allergens and to evaluate the implications of molecular sensitization profiles on respiratory symptoms.* ***Methods.*** *155 consecutive HDM-allergic patients (mean age: 27.5 years; range: 1-62; female: 63), 86 affected by rhinitis and 68 by asthma, were enrolled. Specific IgE reactivity to Der f 1, Der p 1, Der f 2, Der p 2, Der p 5, Der p 7, Der p 10, Der p 11, Der p 20, Der p 21 and Der p 23 was tested in patients' sera using the last version of the multiparametric assay Allergy Explorer[2] (ALEX[2]).* ***Results.*** *In all, major and minor allergens were positive, respectively, in 96.8% and 50.9% of the patients. Prevalence and IgE levels of Der f 1, Der f 2, Der p 1 and Der p 20 were significantly higher in asthmatic patients ($p < 0.05$), whereas subjects negative for minor allergens resulted more frequently suffering from rhinitis ($p = 0.0001$). Asthmatic patients had IgE reactivity to a larger number of HDM allergens (mean 5.4; SD ± 2.3) than patients with only rhinitis (mean 4.2; SD ± 2.5) ($p = 0.003$), whereas no differences in the number of HDM positive molecules and in the specific IgE levels were found among different ages.* ***Conclusions.*** *This study confirms that the assessment of IgE to a comprehensive panel of HDM allergens defines different serological reactivity profiles that seem associated with different clinical presentations.*

Introduction

House dust mites (HDM) are among the most important allergen sources worldwide representing a major cause of perennial allergic rhinitis and asthma. Up to 85% of asthmatic patients are sensitized to *Dermatophagoides (D.) pteronyssinus* and/or *D. farinae* (1), which are the two most important HDM species (2) present in human habitats around the world. Since 1980, when the first allergen of *D. pteronyssinus* was described (3), many other HDM allergens have been identified; some are not only immunogenic but have also proteolytic and immunomodulatory activity (4). Thirty-six allergens for *D. farinae* and thirty allergens for *D. pteronyssinus* have been detected so far (www.allergen.org), even if only group 1 (Cysteine protease: Der p 1, Der f 1), group 2 (NPC2 protein family; Der p 2 and Der f 2) and Der p 23

(Peritrophin-like protein) represent the major and serum-dominant allergens (5). However, other minor allergens such as Der p 4 (α-amylase), Der p 5 (unknown biochemical function), Der p 7 (Lipid-binding protein), and Der p 21 (unknown biochemical function) seem clinically relevant (5, 6). Most allergen extracts obtained from natural sources contain mainly group 1 and group 2 allergens, whereas other important molecules are present in small amounts or are missing, as shown in extracts for skin testing (7). This may have some important consequences not only for diagnosis but also for immunotherapy, since we can speculate that patients with different profiles of IgE reactivity to HDM may respond differently to immunotherapy, as recently shown by Rodriguez-Dominguez *et al*. (8). In addition, other authors, using a comprehensive panel of HDM allergens, revealed that some serological reactivity profiles might help to discriminate asthmatic and non-asthmatic children or might be able to predict the development of asthma (6, 9, 10).

Until now, only group 1 and 2, Der p 23 and Der p 10 (tropomyosin) allergens have been available for the HDM component resolved diagnosis (CRD) in the clinical practice. A more extended panel of HDM allergens using the immunoCAP ISAC technology has been available for research use only. Recently, a new multiparametric assay containing an extended panel of HDM allergens (including Der f 1, Der p 1, Der f 2, Der p 2, Der p 5, Der p 7, Der p 10, Der p 11 (paramyosin), Der p 20 (arginine-kinase), Der p 21, Der p 23) was launched on the marked and can be used in daily practice for HDM CRD.

Our study aimed to evaluate, the prevalence of IgE responses towards this comprehensive panel of HDM allergens and to evaluate the implications of molecular sensitization profiles on the respiratory symptoms (rhinitis and asthma) in a cohort of Italian HDMs allergic patients.

Materials and methods

Patients

155 consecutive HDM-allergic patients (mean age: 27.5 years; range: 1-62; female: 63), diagnosed by a clinical history of perennial rhinitis and/or asthma and positive skin prick tests (SPT) (Stallergenes, Antony, France) and/or *in vitro* assay (Thermofisher Diagnostics, Uppsala, Sweden) with extracts of both *D. pteronisyinus* and *D. farinae,* were enrolled in two allergy Units (Pordenone and Rome) from June 2019 to March 2020. Eighty-seven (mean age: 26.15 years; range: 8-48; female: 36) were affected by rhinitis, whereas 68 were affected by asthma (mean age: 25.5 years; range: 1- 62; female 26) associated (58; 85.3%) or not (10; 14.7%) to rhinitis. Following the GINA guidelines (11), 53 subjects (77.9%) were classified as having mild asthma and 15 (22.1%) as having moderate/severe asthma. No patients underwent to previous HDM specific immunotherapy or declared previous reactions to crustaceans or shellfish.

In vitro assays

IgE specific for Der f 1, Der p 1, Der f 2, Der p 2, Der p 5, Der p 7, Der p 10, Der p 11, Der p 20, Der p 21 and Der p 23 were tested in sera of all patients using the last version of the multiparametric assay Allergy Explorer^2 (ALEX^2) (Macro Array Diagnostics, Wien, Austria). In this system, the allergens are spotted onto a nitrocellulose membrane in a cartridge chip, which is incubated with 0.5 mL of a 1:5 dilution of serum under agitation. After two hours of incubation, the chip is extensively washed and a pre-titred dilution of anti-human IgE labelled with alkaline phosphatase is added and incubated for 30 minutes. Following further washing, the enzyme-substrate is added, and after eight minutes the reaction is completed. The membrane is dried and the intensity of color reaction for each allergen is measured by a coupled-charged device camera. A dedicated software digitalizes the images and produces a report listing components and they score in kUA/L (range 0.3-50 kUA/L). Values above 0.35 kUA/L were considered positive.

IgE specific for extracts of both *D. pteronissinus* and *D. farinae* were also measured using the monoplex ImmunoCAP assay (Thermofisher Diagnostics, Uppsala, Sweden), following the manufacturer's instructions. Values above 0.35 kUA/L were considered positive.

All tests were performed during routine care, and the samples were anonymized, since no personal data, except for age and sex, was available. The Institutional Review Board of IDI-IRCCS confirmed that ethical approval was not required in this case (n. 493.1).

Statistical analysis

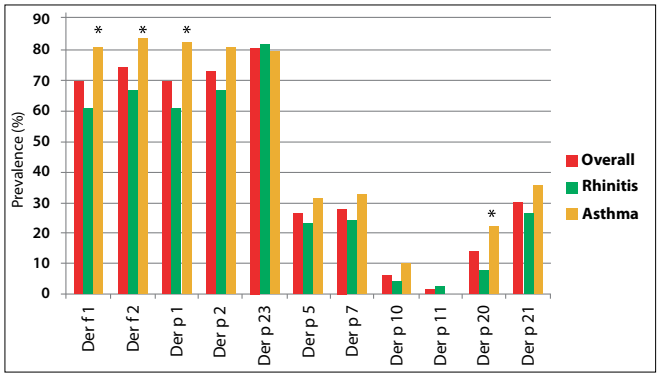
The prevalence of IgE sensitization to the 11 different HDM molecules was evaluated in all subjects and separately in subjects with only rhinitis and with asthma. Differences in specific IgE prevalence between the groups were compared by χ^2 test for categorical variables. Differences of IgE levels between groups were analyzed with the Mann-Whitney U test. A P-value < 0.05 was considered statistically significant. All statistical analysis was performed using MedCalc statistical software, version 10.4.5 (Mariakerke, Belgium) and GraphPad Software (La Jolla, CA).

Results

150 out of 155 evaluated patients were positive for at least one on the 11 HDM allergens, whereas five patients, positive on SPT or on *vitro* test with extracts of *D. pteronissinus* and/or *D. farinae*, were negative for all the tested molecules. No patient negative to the major allergens reacted to the minor allergens. The prevalence of IgE reactivity to individual HDM allergens in all subjects tested, and in subjects with rhinitis and with asthma is shown in **figure 1**. With the only exception of Der p 23, the prevalence of positive results was higher in subjects with asthma, but only Der f 1, Der f 2, Der p 1 and Der p 20 reached statistically significant levels.

Odds ratio (OR) analysis showed that the risk of being asthmatic was more than 2-fold higher in patients in the presence of IgE reactivity to Der f 1 (OR 2.71; 95% CI: 1.20-5.70; p = 0.008), Der f 2 (OR 2.59; 95% CI: 1.18-5.68; p = 0.017) and Der p 1 (OR 2.71; 95% CI: 1.29- 5.70; p = 0.008), and more than 3-fold higher in patients with IgE reactivity to Der p 20 (OR 3.23; 95% CI: 1.23- 8.46; p = 0.001). In contrast, comparing patients with mild and moderate/severe asthma, only reactivity to Der p 21 was significantly associated with moderate/severe asthma (mild asthma: 20%, moderate/severe asthma: 46.4%; p < 0.05), with a 3-fold higher risk of having moderate/severe asthma in Der p 21 positive subjects (OR 3.46; 95% CI: 1.18-10.14; p = 0.02).

Figure 1 *- Prevalence of IgE positivities to individual HDM allergens in subjects with rhinitis (green columns), with asthma (yellow columns) and in combined groups (red columns).*



*Statistically significant differences (p < 0.05).

Concerning the IgE reactivity to major allergens, number and percentage of positivity for a single group of HDM allergens and their combination are shown in **table I**. Interestingly, the presence of the mono-sensitization to Der p 23 was more frequent in subject with rhinitis (p < 0.05).

On the other hand, the minor allergens Der p 5, Der p 7, Der p 10, Der p 11, Der p 20 and Der p 21 resulted positive respectively in 26.4%, 27.7%, 6.45%, 1.3%, 14.2%, and 30.3% of all the patients evaluated, and in 23.0%, 24.1%, 3.5%, 2.35, 8.0% and 25.4% of the patients with rhinitis, and in 30.9%, 32.4%, 10.3%, 0%, 22.1% and 35.3% of the patients with asthma. Patients negative for minor allergens had a probability of 63% of having rhinitis and of 37% of having asthma (p = 0.0001), whereas positive patients had a higher probability of having asthma (52.5%) than negative patients (37%) (p < 0.05) (**figure 2**). 43.5% and 60% of patients with rhinitis and asthma were positive to at least 1 minor allergen (p < 0.05), 24.7% and 41.4% to more than one minor allergen (p < 0.05), and 3.5% and 7.14% to more than 3 minor allergens (p = ns), respectively. In the last case, the absence of statistical significance is probably due to the low number of positive cases.

Concerning allergen specific IgE levels, they resulted higher in asthmatic than in non-asthmatic patients (**figure 3**), but only for Der f 1, Der f 2, Der p 1, Der p 2 and Der p 20 a significant level was reached (p < 0.05). No significant differences in the percentage of positivity and in the IgE specific levels were found comparing patients of different age classes (< 15 years, n = 33; 16-25 years, n = 44; 26-45 years, n = 55; > 45 years, n = 23), as well as no differences in the number of HDM allergens reactivity were shown (**figure 4**). On the contrary, asthmatic patients had IgE reactivity to more HDM allergens (mean 5.4; SD ± 2.3) than patients with only rhinitis (mean 4.2; SD ± 2.5) (p = 0.003) (**figure 4**).

Table I *- Number and percentage of sensitization to the major HDM allergens and their combinations.*

Molecules	Overall n = 155	Rhinitis n = 87	Asthma n = 68	Rhinitis *vs* Asthma
Der p 1$^+$/Der f 1$^+$	104 (67.1%)	51 (58.6%)	53 (77.9%)	p = 0.018
Der p 2$^+$/Der f 2$^+$	114 (73.5%)	58 (66.7%)	56 (82.4%)	p = 0.043
Group 1 + 2	131 (84.5%)	68 (78.2%)	63 (92.6)	p = 0.025
Group 1 alone	14 (9.0%)	8 (9.2%)	6 (8.8%)	p = ns
Group 2 alone	19 (12.3%)	13 (14.9%)	6 (8.8%)	p = ns
Group 1 and 2 + Der p 23	150 (96.8%)	84 (96.6%)	66 (97.1%)	p = ns
Der p 23 alone	18 (11.6%)	16 (18.4%)	3 (4.4%)	p = 0.016
Der p 1$^+$/Der f 1$^-$	3 (1.9%)	2 (2.9%)	1 (1.2%)	p = ns
Der f 1$^+$/Der p 1$^-$	4 (2.6%)	2 (2.3%)	2(2.9%)	p = ns
Der p 2$^+$/Der f 2$^-$	0 (0%)	0 (0%)	0 (0%)	-
Der f 2$^+$/Der p 2$^-$	1 (0.6%)	0 (0%)	1 (1.5%)	p = ns
All negative	5 (3.2%)	3 (3.4%)	2 (2.9%)	p = ns

Figure 2 *- Percentage of subjects with rhinitis (green columns) and asthma (yellow columns) with or without reactivity to HDM minor allergens.*

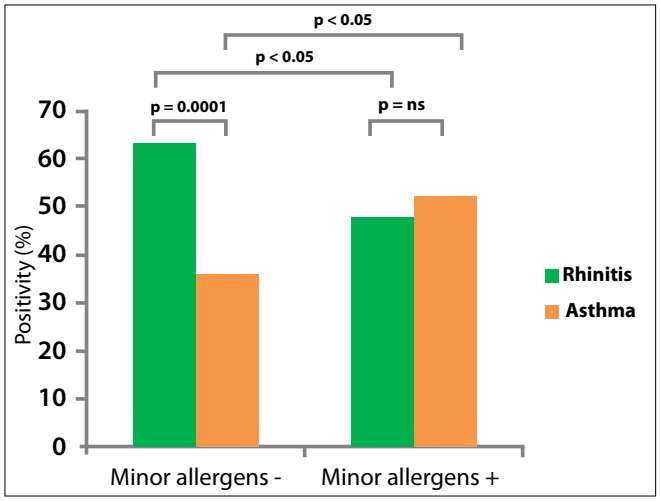
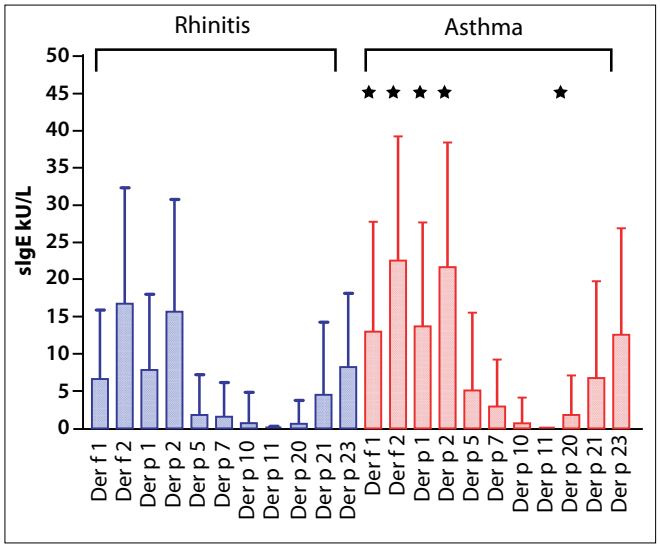


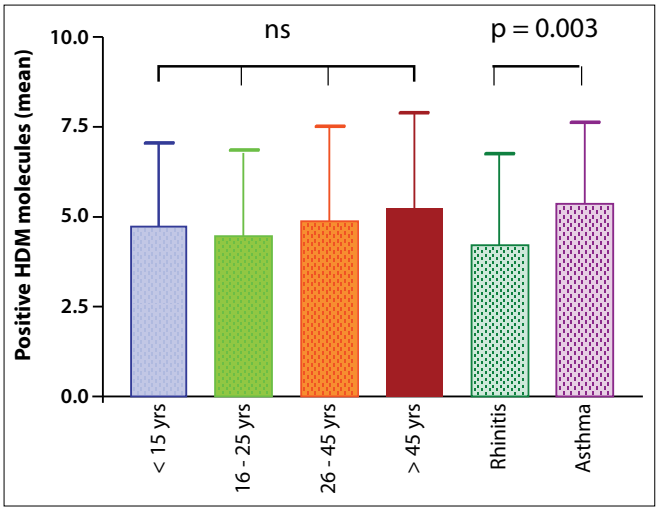
Figure 3 *- Comparison of allergen-specific IgE levels in subjects with rhinitis (blue columns) and with asthma (red columns).*



*Statistically significant difference ($p < 0.05$).

Finally, the level of IgE to extract of *D. ptenonissinus* was strictly related to the le level of Der p 1 ($r = 0.916$, $p < 0.0001$) and Der p 23 ($r = 0.870$; $p < 0.0001$). Lower were the correlations with Der p 5 ($r = 0.504$; $p < 0.0001$), Der p 7 ($r = 0.696$; $p < 0.0009$) and with Der p 21 ($r = 0.690$, $p = 0.003$). Similarly, the level of IgE to extract of *D. farinae* was strictly related to the level of Der f 1 ($r = 0.832$; $p < 0.0001$) and Der f 2 (0.777; $p < 0.0001$).

Figure 4 *- Sum of IgE reactivity to HDM allergens (mean) in different age classes and in subjects with rhinitis only and with asthma.*



ns = p no significant.

Discussion

Our study confirms that among the HDM allergens, group 1, group 2 and Der p 23 are the most important ones in term of prevalence in keeping with other studies of literature (6, 12-16). Most patients show IgE reactivity to group 1 and/or group 2 allergens from both *D. pteronyssinus* and *D. farinae*, but similarly to the data obtained by Batard and coworkers (15) about 5% of them have reactivity to only one of those mite species, confirming that despite a well-known amino acid sequence homology, each of the group 1 (Der p 1 and Der f 1) and Group 2 (Der p 2 and Der f 2) molecules bears species-specific IgE epitopes. This may have some practical consequences both in the diagnostics and in the preparation of extracts for immunotherapy. Der p 23 scored positive in a high percentage of HDM sensitized patients (about 80%), and in 11.6% it was the only positive allergen, a value slightly exceeding that observed in a previous Italian study (16). However, as a difference from the study by Celi and coworkers (16), in which Der p 23 monosensitized patients were more frequently affected by asthma and in particular by severe asthma, in our study Der p 23 monosensitized patients showed a higher prevalence of rhinitis. These differences could be explained by the smaller size of our HDM sensitized cohort, as well as differences in the patients' selection and in co-sensitization to allergens different from HDM (data not evaluated). On the other hand, however, results of our study agree with those of the Manchester Asthma and Allergy Study (MAAS) (9) where children with a more complex molecular pattern of IgE sensitization showed the highest risk of asthma and a significant

higher level of exhaled nitric oxide. Anyway, further studies in large cohorts of HDM hypersensitized subjects are needed to confirm our findings. Considering the other major allergens, the prevalence of Der p 1, Der f 1 and Der f 2 was significantly higher in asthmatic than in nonasthmatic subjects, as well as their specific IgE levels. Also, specific IgE Der p 2 levels were higher in asthmatic patients. These data confirm the results obtained by Resch and coworkers (6).

The good correlations between group 1, group 2 and Der p 23 allergens IgE levels and the IgE levels to extracts *of D. pteronyssinus* and *D. farinae,* confirm that these allergens are not only seroprevalent but also serodominant, representing allergens that quantitatively make the most important contribution to HDM IgE response (5).

Nevertheless, the most interesting novelty offered by the new multiparametric assay used in this study is the possibility to evaluate in daily practice also the IgE responses toward some minor HDM allergens. In the evaluated subjects, 79/155 (50,9%) scored positive for at least 1 minor allergen with a higher prevalence for Der p 5 (overall 26.4%), Der p 7 (27.7%), Der p 20 (14.2%) and Der p 21 (30.3%); these percentages are very similar to those obtained in previous studies using other diagnostic methods (6, 15). Instead, the rate of IgE reactivity to Der p 10 and Der p 11 was very small (6.45%, and 1.3%, respectively). The differences in IgE responses to the minor allergens evaluated might be explained by different routes of sensitization, since Der p 5, Der p 7, Der p 20 and Der p 21 are mainly present in faecal particles and sensitize preferentially through the respiratory tract, whereas Der p 10 and Der p 11 are mainly present in HDM body and therefore may sensitize through the skin or the gut in the case of Der p 10 because of the cross-reactivity with homologous food allergens. It is interesting to remark that some authors reported specific IgE to Der p 11 up to 60% of cases in subject with atopic dermatitis (17), thus representing a major allergen in this clinical condition. However, as we did not consider the co-presence of atopic dermatitis, we cannot confirm these findings. Anyway, the availability of an assay including Der p 11 makes it now possible to design studies on large cohorts of atopic dermatitis subject to confirm the role of this allergen. The prevalence and the level of specific IgE to Der p 5, Der p 7, Der p 20 and Der p 21 were higher in asthmatic patients, though the difference was significant only for Der p 20. Otherwise, the prevalence of Der p 21 resulted significantly higher in the moderate/severe asthma group than in mild asthma. These results are partially different from those obtained by Resch and coworkers (6), who reported that Der p 5 and Der p 7 are more often recognized in asthmatic subjects. However, they did not test Der p 20. Differences between the two studies could be explained by differences in the patients' selection (children in the study of Resch *et al*. and prevalent adults in our study). However, both the studies showed that asthmatic patients with HDM allergy recognize a larger spectrum of molecules, whereas sensitization to fewer components is more related to rhinitis, suggesting that polysensitization to HDM allergens might have functional consequences.

Finally, no differences in the number of HDM positive molecules and in specific IgE levels were found among the different age classes confirming the results of the longitudinal study of Posa *et al*. (10), showing that during the first decade of life the IgE response to HDM components seems to show plasticity, whereas afterwards IgE recognition profiles are more established. However, some limits of this study have to be underlined. First of all, sample size is quite small for a final conclusion on the prevalence of sensitization profiles and their association with diseases and severity of symptoms. Secondly, the observational design of the study implies weaker and less standardized selection criteria.

Conclusions

The results of this study confirm that the assessment of IgE responses toward a comprehensive panel of HDM allergens defines different serological reactivity profiles that seem associated with different clinical presentations. The recent availability in the daily practice of a multiplex assay, able to detect specific IgE toward 11 different HDM components, will allow design large real-life studies to confirm the role of different serologic HDM profiles both in predicting the clinical evolution and the outcome of specific immunotherapy.

Fundings

None.

Conflict of interests

The authors declare that they have no conflict of interests.

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Probiotic as an adjuvant therapy in chronic urticaria: a blinded randomized controlled clinical trial

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Key words

Chronic spontaneous urticaria; probiotic; prebiotic; symbiotic; antihistamine; RCT; UAS score; DLQI; urticaria; CSU; trial.

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Impact statement

Based on UAS7 score, combination of probiotic + antihistamines had no statistically significant different efficacy than antihistamine alone, associating with higher reduction rate of itch, number of urticaria and total UAS7 score.

Summary

Background. *Chronic spontaneous urticaria (CSU) is a common and treatment challenging disorder which may involve about 2% of normal population and in 50% do not respond properly, even to the second line therapies. We aimed to evaluate the efficacy and safety of a symbiotic (prebiotic + probiotic) named as Lacto-Care in treatment of CSU in the RCT for the first time.* ***Methods.*** *This blinded RCT conducted on 42 patients (21 patients in control antihistamine group and 21 in intervention antihistamine + probiotic group) with CSU during 8 weeks. The efficacy was assessed by Urticaria Activity Score (UAS7) and quality of life measured by Persian validated Dermatology Life Quality Index (DLQI).* ***Results.*** *Before and after, in control group UAS7 score was 35.33 ± 7.81 and 16.86 ± 13.54, respectively. There was 53% score reduction in control group. Before and after, in intervention group UAS7 score was 32 ± 7.84 and 11 ± 11.41, respectively. There was 66% score reduction in intervention group. In control and intervention group improvement of DLQI was 44% and 66%, respectively. At the end, UAS7 score reduction and DLQI improvement was statistically significant in both groups.* ***Conclusions.*** *Probiotics are effective, safe and satisfactory adjuvant therapy for CSU. Combination of probiotic and antihistamines had no statistically significant different efficacy than the antihistamine alone, based on UAS7 score. But patients with combination therapy may experience higher reduction rate of itch, number of urticaria and total UAS7 score that is clinically of great value and is really practical by itself. Patients with combination therapy experienced more improvement of quality of life (DLQI).*

Introduction

It seems that up to 20% of individuals at some point in their life may be affected by urticaria and angioedema. Episodes lasting for less than 6 week are considered acute, whereas those occurring on most days for more than 6 week are considered as chronic urticarial (CU). The etiology, mechanism, causes and therapeutic options are different in acute and chronic urticarial; therefore, the distinction is considered of great importance. Acute urticaria is a self-limited condition, and mast cell activation with an allergen, the main activator. Moreover, foods, drug, insect venom or sting and viral infections are the main causes (1-8).

Chronic urticaria has significant burden and great impact on patient's quality of life and is associated with much psychological comorbidity (9-11). The most common form of management and treatment system of chronic urticaria is symptomatic therapy. In 5 to 50 percent of patients with chronic urticaria, the first-line treatment (one type of antihistamine regimen) may not result in satisfying disease control. Patients with refractory chronic urticaria require a 4-fold increase in the H1 antagonist dose or continue treatment with omalizumab, cyclosporine, or montelukast if urticaria persists. In patients with chronic spontaneous urticaria (CSU) resistant to four-fold usage dose of antihistamines with higher dose consumption, only 49% of patients reported a decrease or resolution of symptoms, and in 20% of patients, side effects were appeared (12-16).

Studies have been shown that *Lactobacillus salivarius* LS01 and compound of 2 probiotics (*Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03) are capable of producing and releasing pro-Th-2 cytokines from Th-1 cells, and help to improve T-helper cells type Th1/Th2 (17-19).

Probiotics are viable microorganisms that have beneficial effects on the body when consumed in sufficient quantities, and as their great feature, they are safe and secure for the host (20).

Probiotics have been studied with promising results in the treatment of atopic dermatitis (AD), acne, eczema, allergic diseases, skin aging, bacterial and fungal infections, chronic wound healing like diabetic foot ulcers (21-26).

Th-2 cells play a critical role in the pathogenesis of allergic reactions and production of urticarial-related antibodies.

The high prevalence of CU and its great effects on the quality of life of patients and their family as well as the partially failure of current treatments led us to design and implement the following study.

Based on our literature review, this is the first blinded RCT to evaluate the efficacy and safety of symbiotic for treatment of chronic urticaria.

Materials and methods

The present interventional study was a parallel study; analyst blinded randomized controlled clinical trial conducted on 42 patients with chronic urticaria at the allergy and dermatology department of Rasool Akram Medical Complex of Iran University of Medical Sciences, between February and December 2019. Sample size was calculated according to the study of Nettis *et al.*, (16) with regard to P1 = 71.1, P2 = 28.9, α = 0.05 and power = 90%, then, 36 patients needed to be enrolled in the study. Fifty-two patients were enrolled but 10 of them discontinued the study and finally 42 patients finished (each group: 21 patients).

Eligibility criteria

Patients with chronic non-autoimmune non-vasculitic urticaria (at least two days a week more than 6 weeks), aged 18 to 45 years, without any serious co-morbidities (such as malignancies, mental illness, hepatitis, endocrine, rheumatologic or other acute and chronic systemic diseases), not treated with any drugs other than antihistamines, no history of acute gastrointestinal illnesses such as indigestion and mal-absorption and not taking any corticosteroids for any reason were included in the study.

Patient recruitment

Informed consent was filled prior to recruitment. Liver, thyroid, kidney function in addition to peripheral cell analysis and autoimmunity screening was done. Assays before allocation is listed as: CBC Diff, BUN/Cr, ALT, AST, ALP, ANA, RF, ESR, CRP, TSH, ANTI TPO, U/A, S/E, stool *H. pylori* Ag, and in the case of abnormality detection due to probability of systemic disorder, they did not enrolled in the study. The recruitment phase was finished within 5 months. It should be notified that patients were free to exit the study at any time by any reasons such as unwillingness to continue the study or due to side effects especially which did not respond to routine approaches including dose reduction or changing drug type.

Random sequencing and allocation

Patients were assigned to control (only antihistamine) and intervention groups (antihistamine + symbiotic) through computerized randomization with a 1:1 allocation ratio.

Follow-Up and evaluation

Study duration was 8 weeks with 2 visits as the first day visit and final visit after 8 weeks. The safety profile for the probiotic strain over 8 weeks of treatment in our patients was consistent with previous observations in patients treated with probiotic supplement (27). Questionnaire 1 was completed for all eligible participants at the beginning of the study including demographic data of patients (age, sex, occupation, weight, marital status, urticaria number, severity, duration, characteristics and its triggering factors, number of involved days per week, previous antihistamine use and its type). By supervision of the main study investigator, in questionnaire 2 and 3, patients recorded the number and severity of urticaria based on Urticaia Activity Score (UAS7) questionnaire (28) and their quality of life based on Persian validated Dermatology Life Quality Index (DLQI)

questionnaire (29), respectively. The number and severity of urticaria and also quality of life (questionnaires 2 and 3) were recorded by patients in 8th week visit, again.

Detailed information of questionnaires

Questionnaire 2 filled based on the patient's condition at last week which was designed in two columns, the first column was for itch: 0 (no itching), 1 (slight no annoying itching), 2 (medium annoying itching but does not interfere with daily activities), 3 (severe itching that is annoying and interfere with sleep or daily activities) and the next column was related to the number and severity of urticaria: 0 (no lesion), 1 (less than 20 lesions during 24 hours), 2 (20-50 lesions during 24 hours), 3 (over 50 lesions during 24 hours or a large area of the body involved by large interconnected lesions) (28). *UAS7 score = (Itch score + Urticarial number score) × (number of involved days per week).* Range (0-42), where 0 = no lesion, 1-6 = well controlled, 7-15 = mild, 16-27 = moderate, 28-42 = severe. Based on previous studies, at the end of study, the percentage of UAS7 score reduction was calculated and a ≤ 10%, 11-30%, 31-90% and > 90% score reduction was considered as no response, mild response, significant response and complete response, respectively. DLQI score: 10 item. Range (0-30) (29).

Blinding

There was no blinding of patients or investigator in the study so that only data analyst was blind.

Treatment regimens

Among these three oral antihistamines (cetirizine 10 mg, desloratadine 5 mg and fexofenadine 180 mg), two of them were selected and given twice a day (for example cetirizine + desloratadine or desloratadine + fexofenadine, *etc*.). The selection criteria were based on the patient's preference and appropriate previous clinical response. This treatment strategy was the same for both groups. But in intervention group, in addition to similar antihistamines regimen, patients received twice daily oral probiotic capsules named LactoCare, manufactured by Iranian Bio Fermentation Company. LactoCare capsule is a synbiotic (probiotic + prebiotic), which contains high amounts of many beneficial and safe bacterial strains (*Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, *Streptococcus thermophilus*) plus fructooligosaccharides as prebiotic.

Laboratory tests

CBC Diff, BUN/Cr, ALT, AST, ALP, ANA, RF, ESR, CRP, TFT, ANTI TPO, U/A, S/E, stool *H. pylori* Ag, test only at the beginning of the study for all participants.

Statistics and data analysis

Descriptive results for values are presented as mean ± SD or percent. Independent t-test and Mann-Whitney test were used to compare the two means. Cochran test was used to investigate the differences between the binary qualitative variables during the measured time. Repeated measures ANOVA and LSD were used to evaluate the quantities over time and between groups. P-values less than 0.05 were considered statistically significant. All data were analyzed using SPSS 21 software as intention to treat.

Ethics and study registration

All stages of this research are committed to the Helsinki Declaration and all patients' information is protected. The ethical code of this study was: IR.IUMS.FMD.REC.1398.129 and the IRCT number was: IRCT20190825044613N1.

Results

The present study was performed on 42 patients with chronic spontaneous urticaria (21 patients in intervention symbiotic antihistamine group and 21 patients in control antihistamine group). At first, among demographic data, only the mean WBC count and lymphocyte percentage in the intervention group was significantly lower and higher than the control group, respectively (6800 *vs* 8400 p = 0.047; 46% *vs* 37% p = 0.038) (**table I**).

The mean UAS7 score was not statistically different between groups, before therapy. Although after therapy, a statistically significant score reduction was observed in both treatment groups (p < 0.001) and this score reduction was higher in intervention group, with using the independent t-test, final mean UAS7 score and reduction rate of UAS7, were not significantly different between treatment groups (p > 0.05) (**table II**). There was not any statistically significant difference between two groups regarding the type of antihistamines regimen and the final UAS7 score (p > 0.05). The severity of itch, at first and after 8 weeks of therapy was evaluated and the results showed that at the end of the study the itch severity had a significant decreased in both control and intervention group (p = 0.047; p < 0.001, respectively). Itching severity was significantly lower in the intervention group than the control (p < 0.05), but rate of itching decline during the study, was not statistically different between two groups (p = 0.162).

The urticaria number, at first and after 8 weeks of therapy was evaluated and the results showed that at the end of the study, the urticaria number had a significant decreased in control and intervention group (p = 0.001; p < 0.001, respectively). The urticaria number was significantly lower in the intervention group than the control (p < 0.05), but the rate of urticaria number decline during the study, was not statistically different between two groups (p = 0.073).

Based on UAS7 categories, in the control group, firstly 15 (71.42%) and 6 (28.57%) of the patients had severe and moderate CSU, respectively which at the end of the study changed to 6 (28.57%), 2 (9.52%), 2 (9.52%) and 11 (52.38%) as sever, moderate, mild and well-controlled CSU, retrospectively.

Based on UAS7 categories, in intervention group, firstly 19 (90.47%) and 2 (9.52%) of the patients had severe and mod-

Table I *- Comparison between control and intervention group regarding basic demographic characteristics.*

Variable	Group	Mean	SD	P-value
Age (year)	Control	40.21	13.74	
	Intervention	36.50	10.75	0.352
Weight (kg)	Control	73.31	11.17	
	Intervention	67.37	14.06	0.181
WBC (count)	Control	8376.11	2164.57	
	Intervention	6830.38	2529.42	0.047
Hb	Control	13.09	1.79	
	Intervention	13.56	2.16	0.564
Neutrophil (%)	Control	50.21	12.36	
	Intervention	46.58	14.43	0.418
Lymphocyte (%)	Control	37.15	12.19	
	Intervention	45.94	12.16	0.038
Eosinophil (%)	Control	3.62	2.57	
	Intervention	3.02	2.39	0.483
Urticaria duration (week)	Control	19.81	50.661	0.931
	Intervention	18.76	22.430	
Days involved with urticaria per week (day)	Control	6.43	2.014	0.729
	Intervention	6.24	1.480	
		-	**+**	**P-value**
H. pylori (%)	Control	14 (66.7)	7(33.3)	0.215
	Intervention	9 (42.9)	12(57.1)	
		-	**+**	**P-value**
ANA (%)	Control	20 (95.2)	1(4.8)	> 0.05
	Intervention	20 (95.2)	1(4.8)	
		Female	Male	**P-value**
Gender (%)	Control	15 (71.4)	6(28.6)	0.633
	Intervention	15 (71.4)	6(28.6)	
Previous therapeutic regimens (%)		**Steroids**	**Steroids + Antihistamines**	**P-value**
	Control	11 (52.4)	10(47.6)	0.197
	Intervention	16 (76.2)	5(23.8)	
Types of prescribed antihistamines regimens (%)		**Cetirizine + Fexofenadine**	**Cetirizine + Desloratadine**	**P-value**
	Control	11 (52.4)	10(47.6)	> 0.05
	Intervention	11 (52.4)	10(47.6)	

erate CSU, respectively, which at the end of study changed to 3 (15.78%), 4 (19.04%), 7 (33.33%) and 7 (33.33%) as sever, moderate, mild and well-controlled CSU, retrospectively.

So, in the control group, 3 (14%), 3 (14%), 11 (53 %) and 4 (19%) of the patients had no, mild, significant and complete response, respectively, and in the intervention group 2 (9%), 12 (58%) and 7 (33%) of the patients had mild, significant and complete response, respectively. In overall, 19% and 33% of the patients in two groups, respectively had complete therapeutic response.

At the baseline of the study, there was no significant difference between groups regarding distribution of prescribed antihistamines combination regimen ($p > 0.05$) and by χ^2 test it was showed that this regimen did not have a confounding effect on the study results.

In **table III** you can see the mean final patients' quality of life which increased significantly in both treatment groups and in intervention group, quality of life improvement was significantly higher than the control ($p < 0.05$).

In **figure 1**, we showed the main change of UAS7 Score (response) and patients' quality of life, before and after therapy in the control and intervention and group.

We did not find any serious or irreversible side effects among all participants of the study. Some patients had complaint of

Table II *- Mean UAS7 score before and after therapy in both groups and compare the final UAS7 score between the groups.*

	Group	Mean	N	SD	Score reduction (%)	P-value
Control	UAS7 Before	35.33	21	7.81	53%	< 0.001
	UAS7 After	16.86	21	13.54		
Symbiotic	UAS7 Before	32.00	21	7.84	66%	< 0.001
	UAS7 After	11.00	21	11.41		
	Group	**N**	**Mean**	**SD**		**P-value**
Final UAS7 score in 8th week	Control	21	16.86	13.54		0.137
	Symbiotic	21	11.00	11.41		

Table III *- Mean DLQI of patients in control and intervention group before and after therapy and comparing the final DLQI score between 2 groups.*

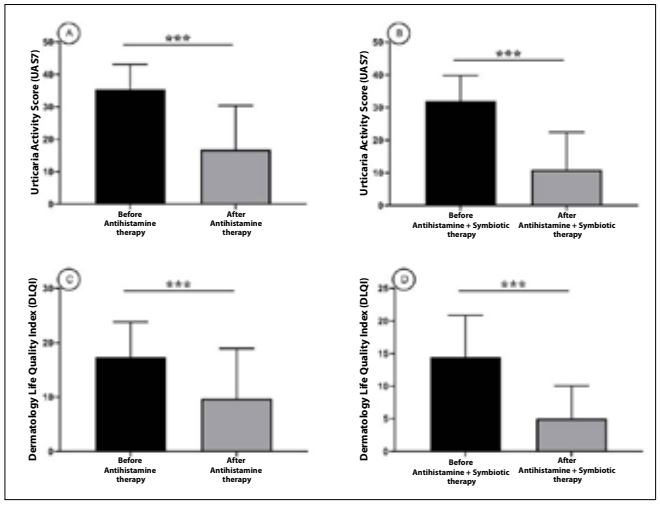
	Group	Mean	N	SD	Improvement of quality of life (%)	P-value
Control	DLQI Before	17.33	21	6.49	44%	< 0.001
	DLQI After	9.71	21	9.24		
Symbiotic	DLQI Before	14.47	21	6.40	66%	< 0.001
	DLQI After	5.04	21	5.00		
	Group	**N**	**Mean**	**SD**		**P-value**
Final DLQI in 8th week	Control	21	9.71	9.24		0.049
	Symbiotic	21	5.04	5.00		

drowsiness and few patients in symbiotic group experienced mild GI discomfort that was not sustainable.

Discussion

Chronic spontaneous urticaria is a common and treatment challenging disorder which may involve about 2% of normal population and in 50% of cases do not respond properly even to the second line therapies (2, 3, 5, 13-16). Regarding these facts, there are many trends to find and evaluate safe therapeutic options with better or additive response rate. The skin is the largest organ in the body that carries hundreds of microorganisms called the skin microbiota. Colonized bacteria react with toll like receptors on the intestinal epithelial cells and dendritic cells which cause activating and signaling of immune cells including macrophages, NK cells, B cells, T helper cells, cytotoxic T cells and regulatory T cells. Intestinal microbiota imbalance can lead to allergic reaction, thus regulating immune system through intestinal microbiota can affect chronic urticaria (17, 18). There is evidence suggesting that alteration of the composition and/or size of the gut microflora may modulate the IgE response to allergens (19). Probiotics are viable microorganisms that have beneficial effects on the body when consumed in sufficient quantities and their great feature is that they are safe and secure for the host (20). Nowadays, there are many promising data regarding the benefits of microbiota regulation and use of prebiotics, probiotic and symbiotic in various fields of medicine including dermatology (21-26). Whereas modern lifestyles have

Figure 1 *- The mean change of UAS7 Score (response) and patients' quality of life, before and after therapy in control and intervention groups.*



contributed to changes in the composition of the intestinal microflora, diet supplementation with probiotics may counterbalance the Th-2 activity by promoting Th-1 cytokines production and down regulate IgE production via inhibition of IL-4 and IL-5 production (28-30). In cases of CSU, in which autoreactive IgG antibodies against FcεRI, IgE, or both or autoreactive IgE antibodies against autoallergens are found, these autoantibodies are causative factors, and IgE, FcεRI, and mast cells are unambiguously at the centre of the pathologic process. For the remaining cases of CSU, IgE, FcεRI, and mast cells are also likely to play essential pathologic roles, although the causative factors have not been identified. Autoimmune processes might be the primary cause of most cases of CSU. Thus, for those cases with a clear autoimmune cause, the reduction of the IgE by the action of probiotics yields the observed therapeutic efficacy. Even for those cases that involve autoimmune response and autoreactive IgE antibodies subtly, they still involve the central pathologic axis of IgE-FcεRI-mast cells, and probiotics similarly render therapeutic effects (31, 32). However, in recent years, several lines of evidence suggest that some bacterial probiotics can modulate the skin immune system (33).

Since there are many evidences regarding the role of microbiota in pathogenesis and course of the CSU specially gut flora, also little case series or trials focusing on this entity, we designed the first RCT to evaluate the efficacy and safety of a symbiotic (prebiotic + probiotics) named as LactoCare in treatment of CSU.

Specific studies about effects of microbiota and symbiotics in course and treatment of chronic urticaria are really rare, so we discuss about the role of symbiotics in other dermatoses at first and then discuss about urticaria with more detail.

In a review study by Notay *et al.* (34), conducted in 2017 entitled "Probiotics, prebiotics, and synbiotics for the treatment and prevention of adult dermatological diseases" the results indicated that studies were optimistic about the use of some probiotic and prebiotics strains to improve clinical response in symptomatic AD, also as a treatment for acne. In addition, this review emphasis on further research to evaluate how probiotics and prebiotics could be better used in dermatology.

In a study by Rezazadeh *et al.*, (35) from Iran, authors investigated the protective effect of *Lactobacillus* and *Bifidobacterium* against chronic urticaria. In this study, stool samples of 20 patients with chronic urticaria were compared with 20 age- and sex-matched healthy controls in terms of *Lactobacillus*, *Bifidobacterium* and *Bacteroides* contents. The results showed that there was no significant difference between the frequencies of these bacteria between groups.

Another study was conducted in 2017 by Nabizadeh *et al.* (36) to investigate the relationship between microbiota composition and chronic urticaria. In this study, 20 patients with chronic urticaria and 20 age-matched healthy controls were selected. The PCR of bacterial DNA genome results showed that the frequency of *A. muciniphila*, *C. leptum* and *F. prausnitzii* in the stool of healthy subjects was significantly higher than in patients with chronic urticaria ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively).

Nettis *et al.* (2016) (37), conducted 8 weeks clinical trial on 38 patients with severe CSU to evaluate efficacy and safety of probotics. They used UAS7 score as primary outcome measure also assessed the patients' quality of life. Patients received double dose of 3 or more types of antihistamines also received oral probiotics named as Bifiderm twice daily. Patients visited at the beginning, 4 weeks and 8 weeks after study started. Nine patients experienced mild clinical improvement (23.7%), one patient reported significant clinical improvement (2.6%) and one patient completely recovered (2.6%). Twenty-seven patients showed no signs of recovery (71.1%). In addition, no adverse effects were reported during the study. In this study we evaluated the clinical efficacy and safety of an intake of a capsule is a symbiotic (probiotic + prebiotic), which contains high amounts of many beneficial and safe bacterial strains (*Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, *Streptococcus thermophilus*) plus fructooligosaccharides as prebiotic in patients with CSU.

The Nettis *et al.*'s study (37) was a single arm before-after trial, but our study was a RCT; we compared the results of Nettis's study with the results of the intervention arm of ours that showed 2 (9%), 12 (58%) and 7 (33%) of our patients had mild, significant and complete response, respectively. Our positive therapeutic results were more excellent than the previous study and may be due to better case selection, randomization and the least confounders due to better study design, also all cases of Nettis's study initially had sever urticaria with highest UAS7 score but 90% of our CSU group had severe

urticaria with mean UAS7 score as 32. In our study improvement of quality of life was higher than the Nettis's study, logically due to better therapeutic response. The safely was comparable with Nettis's study. In a review article of Ghaffari *et al*. (2013) (38), the efficacy of similar therapeutic regimens has been mentioned, as that our study confirmed too. The DLQI was conversely related to urticarial severity that is to somehow similar to our findings that final DLQI was higher in the intervention group which had higher UAS7 score reduction. Baiardini *et al*. (2003) (39) found that the quality of life in CSU patients were lower than the respiratory allergies in various social, physical and psychological aspects, that is comparable with our results which showed an improvement of DLQI during the study by decreasing of UAS7 score. In our study, although based on the statistical analysis, there were not any significant differences between control and intervention group regarding UAS7 score (final UAS7 score and the reduction rate during the study), but there were many differences in severity category change and response rate of 2 treatment groups during time, that are really important in practical managements of patients. So that symbiotics may make the therapeutic results of urticaria better but for more exact interpretations, we need further studies with higher sample size. There are many articles in dermatology regarding potential role of microbiota and efficacy of prebiotics, probiotics and symbiotic in many dermatoses such as dermatitis, atopic eczema, acne and *etc*., that in this study we focused on chronic urticaria and designed the first blinded RCT, in this regard (40-44).

Conclusions

Probiotics and symbiotics are effective, safe and satisfactory adjuvant therapy for CSU, although combination of probiotic and antihistamine did not have significant efficacy difference compared with the antihistamine alone based on final mean UAS 7 score, but patients with combination therapy may experience higher mean reduction rate of UAS 7 (although insignificant) and also significantly higher reduction rate of itch and number of urticaria, that is clinically really important and practical. In conclusion, our study suggests that probiotics administered twice daily for 8 weeks might reduce the symptom scores and quality of life scores in a part of patients with CSU who remained symptomatic despite treatment with H1 antihistamine. The probiotic approach might represent a new well tolerated option in the treatment of CSU.

Limitations and recommendations

Loss to follow ups was one of the major limitation of this study, also our study was not patient blinded as we did not have any placebo. High cost of symbiotics was another limitation of our study. With higher sample size and well-designed study, it is probable to observe significant difference between routine and combination therapies (+ probiotic or symbiotics), regarding score reduction.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Allergen sensitization associates with worse lung function parameters

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Impact statement

The presence of allergen sensitizations was significantly associated with worse key spirometry parameters, increased bronchodilator response and higher specific resistance.

Summary

Objective. *To assess the association between the number of allergen sensitizations and lung function variables in individuals with airway symptoms.* ***Methods.*** *Retrospective study with all individuals who performed lung function and skin-prick tests at CUF-Porto (01/2011-06/2016). Six allergen groups were considered. % predicted pre-bronchodilator test (BD) and % change after BD were analysed for spirometry and plethysmography parameters.* ***Results.*** *A total of 1293 individuals were included, 54% (n = 698) adults and 69% (n = 891) with sensitization to ≥ 1 allergen group. %FEV1 was significantly higher and % change in FEV1 significantly lower in non-sensitized individuals. %sRaw was higher in polysensitized (vs non-sensitized).* ***Conclusions.*** *The presence of allergen sensitizations was significantly associated with worse key lung function parameters.*

Introduction

Sensitization to common environmental allergens is frequent: it is estimated that around 40% of the worldwide population is sensitized to at least one allergen (1). Allergic sensitization can be asymptomatic (2-4). However, it is a strong predictor for the future development of allergic diseases (like asthma or allergic rhinitis (5, 6)).
The number of sensitizations has been associated with the number of allergic comorbidities, with polysensitization being a strong risk factor for the development of multimorbidity (7, 8). Raciborski *et al.* observed that multimorbidity was rare in the case of negative skin-prick tests (SPT) to common inhalant allergens, more frequent in monosensitized subjects (one positive SPT) and very frequent in those with polysensitization (two or more positive SPT) (7). In recent studies including allergen sensitizations in cluster analysis to classify individuals with airways disease, it was reported that presenting a larger number of allergen sensitizations was associated with classification in clusters with higher disease severity (9, 10).
Considering the relation between polysensitization and multimorbidity and allergic disease severity, we hypothesized that the

number of allergen sensitizations might also be associated with objective parameters of lung function.

Lung function tests (LFT) are an important tool in the assessment of patients with respiratory disease, especially with asthma, but may also be relevant in patients with AR (11). Only a few studies have previously reported an association between atopy and decreased lung function, mainly in individuals with asthma (12, 13). To our knowledge, only one study, including children with asthma, described a significant relation between polysensitization (≥ 4 allergens) and lower FEV1/FVC, but the reported 95% confidence interval suggested a nonsignificant association, raising doubts about the meaning of this result (14). Thus, the association between the number of allergen sensitizations and lung function, irrespective of disease diagnosis, is not well studied yet.

The aim of this study was to assess the association between the number of allergen sensitizations and lung function variables in individuals with airways symptoms.

Materials and methods

Sample and study design

This was an observational, retrospective study with all individuals who performed body plethysmography (BP) or spirometry with or without bronchodilator test (BD), and SPT at the Allergy, Inflammation and Respiration laboratory (part of an Allergy Clinic) at CUF Porto, Portugal, between January 2011 and June 2016. Only the most recent assessment of each individual was included. All data were collected during routine care and the analysis was performed using an anonymised dataset with no personal identifier. Therefore, Ethics Committee approval was not required.

Variables and measurements

Spirometry and plethysmography were performed following the ATS/ERS recommendations (15). BD test was performed, when requested, with 400 μg salbutamol, delivered through holding chamber, with subsequent tests being repeated after 15 minutes. The standard centre protocol for bronchodilator medication before LFT includes a general advice to withhold inhaled medication for at least 12 hours before LFT; nevertheless, the physician requesting spirometry or BP may give a different advice according to the specific indication for testing. These recommendations are in line with the 2005 ATS guidelines for lung function testing (15).

A detailed list of the spirometry and BP parameters that were analysed is presented in **online supplements table IS**.

Allergic sensitization was assessed by SPT, which were performed according to the guidelines of the European Academy of Allergy and Clinical Immunology (16). The standard allergen panel included two controls and 14 allergens that were categorized into six groups: 1) mites; 2) dog and cat epithelia; 3) tree pollens; 4) grass pollens; 5) weed pollens; and 6) molds. Papules were measured by planimetry (Inmunotek prick-film™), scanned and processed using a specific reading software (17). The positivity criterium was the presence of an allergen wheal with > 50% of the histamine wheal area (skin index > 50%) (18, 19). We grouped the individuals according to the number of sensitizations: not sensitized (0), monosensitized (1), polysensitized to 2 groups of allergens (2) and polysensitized to 3 or more groups of allergens (≥ 3). Demographic characteristics, such as age and sex, were also analysed.

Statistical analyses

Categorical variables are presented as absolute frequencies and proportions. Continuous variables were presented using mean and standard deviation (SD).

One-way ANOVA was used to compare lung function parameters among groups of allergen sensitizations (with Bonferroni *post-hoc* test for multiple comparisons); we stratified this analysis by age group (considering children < 18 years and adults ≥ 18 years old). We also used ANCOVA to further explore the impact of age (included as a continuous covariate) in the relation between lung function and the number of allergen sensitizations. The statistical analysis was performed using IBM SPSS Statistics version 25.0 (Armonk, NYIBM Corp). A P-value of < 0.05 was considered statistically significant.

Results

We have included 1293 individuals aged 3 to 86 years old: 447 (35%) under 13 years, 148 (11%) with 13 to 17 years old and 698 (54%) with ≥ 18 years old; 688 (53%) were female. More than two thirds (n = 891; 69%) were sensitized to ≥ 1 allergen group (**table I**).

Spirometry

The description of the spirometry parameters is shown in **online supplements table IIS**. The comparison of spirometry parameters among groups of allergen sensitizations is shown in **figure 1** and **online supplements table IIS**. There were statistically significant differences between some of the groups, most when comparing non-sensitized *vs* groups with at least one sensitization. These differences occurred in %FVC, %FEV1, FEV1/FVC, %MMEF, and %PEF with those in the non-sensitized group presenting higher values than at least one of the other groups. Percent changes in FEV1 and MMEF were significantly lower in the non-sensitized group compared to at least one of those with allergen sensitizations. The comparisons of FEV1 presented the most consistent results, with %FEV1 being significantly higher and the % change in FEV1 being significantly lower in non-sensitized individuals *vs* all groups with sensitizations.

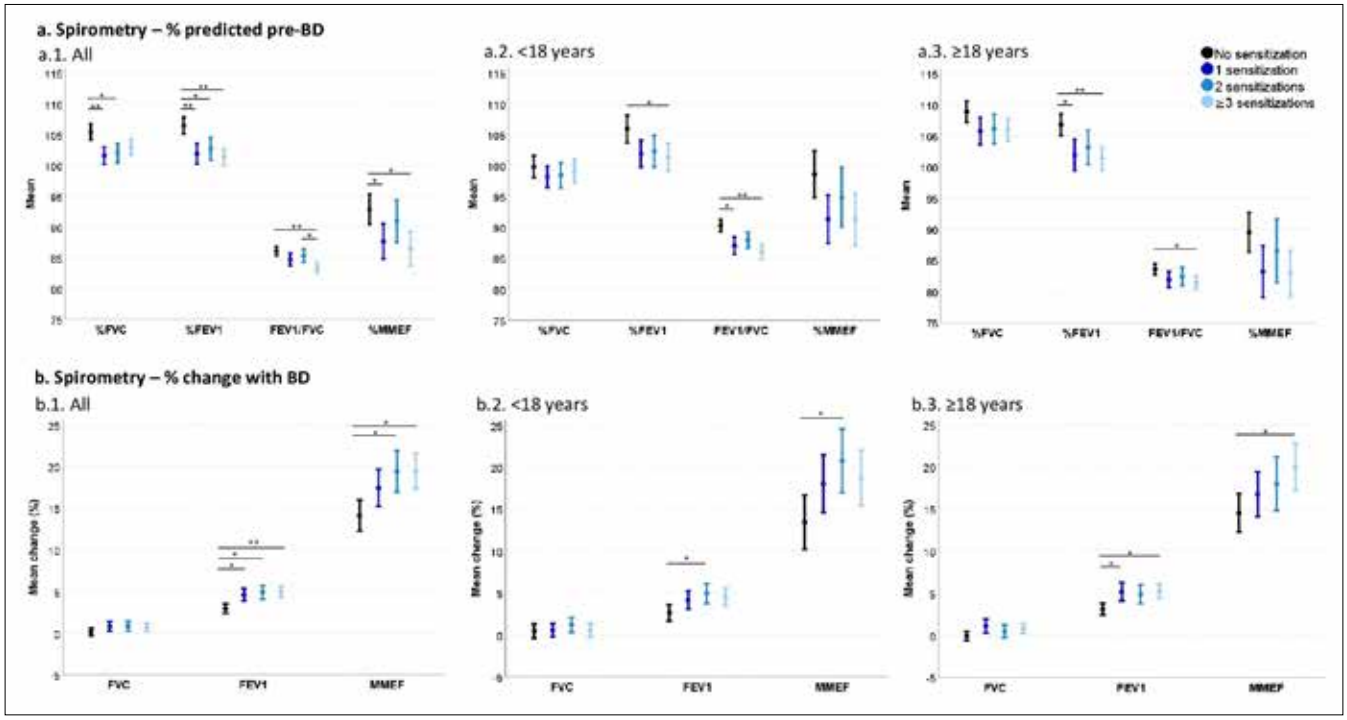
When stratifying by age groups, in children there were no statistically significant differences in FVC between the sensitization groups. The %FEV1 and FEV1/FVC were significantly higher in non-sensitized children *vs* polysensitized (≥ 3), and FEV1/FVC was also higher in non-sensitised *vs* monosensitized (**figure 1** and **online supplements table IIIS**). The % changes in FEV1 and MMEF

Table I *- Description of the study participants.*

	Total* (n = 1293)		**Number of allergen sensitizations**								**Body plethysmography** (n = 287; 22%)	
			0 (n = 402; 31%)		**1** (n = 320; 25%)		**2** (n = 241; 19%)		**≥ 3** (n = 330; 26%)			
	n	%	n	%	n	%	n	%	n	%	n	%
Sex, female	688	53	267	67	150	47	117	49	154	47	151	53
Age group, ≥ 18 years old	698	54	252	63	143	45	113	47	190	58	198	69
Age, mean (SD)	25	(17)	30	(20)	21	(16)	22	(16)	25	(15)	32	(18)
Allergen sensitizations												
Mites	703	55	0		247	77	184	76	272	82	163	57
Epithelia	329	26	0		15	5	78	32	236	72	81	28
Grass pollens	484	38	0		45	14	133	55	306	93	106	37
Tree pollens	266	21	0		6	2	44	18	216	65	69	24
Weed pollens	215	17	0		6	2	30	12	179	54	50	18
Molds	82	7	0		1	0.3	13	6	68	21	19	7
At least one	892	70	0		320	100	241	100	330	100	203	73

*All study participants performed spirometry.

Figure 1 *- Mean with 95% confidence interval for spirometry parameters, including % predicted pre-BD (panel a) and % change with BD (panel b) stratified by age group (1. All; 2. < 18 years; 3. ≥ 18 years).*



*p < 0.05; * p < 0.001.

were significantly lower in non-sensitized children *vs* those polysensitized to 2 allergens (**figure 1** and **online supplements table IIIS**). In adults, spirometry parameters showed significant differences between the groups, except for the % change in PEF. %FEV1 and % change in FEV1 were significantly higher in non-sensitized *vs* monosensitized and polysensitized to ≥ 3 allergen groups. FEV1/FVC and % change in MMEF were significantly higher and lower, respectively, in non-sensitized *vs* polysensitized to ≥ 3 allergen groups (**figure 1** and **online supplements table IVS**). %PEF was also higher in non-sensitized *vs* monosensitized (**online supplements table IVS**). Adjusting for age with ANCOVA led to similar findings (results not shown).

Body plethysmography

Of the 287 BP analysed, 89 (31%) were performed in children (mean (SD) age 10 (3) years) and 199 in adults (42 (13) years; **table I**). In children, 60% were male while in adults, 58% were female.

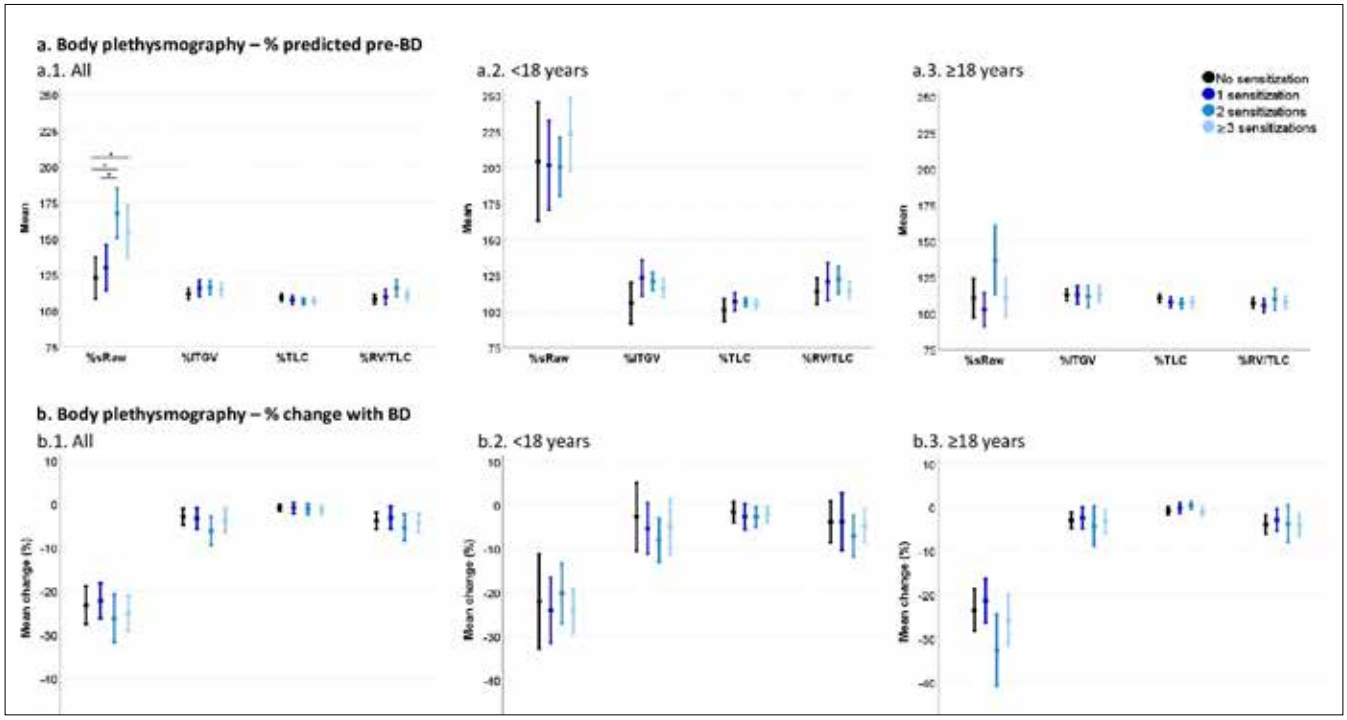
In **figure 2** and **online supplements table VS** are summarized the BP parameters and the stratification and comparison of BP parameters according to the number of allergen sensitizations.

A third of the participants had sRaw > 150%. %sRaw was higher in polysensitized (2 or ≥ 3 sensitizations) *vs* non-sensitized and in those sensitized to 2 allergen groups *vs* monosensitized. No other BP parameter presented significant differences among groups of allergen sensitization. In children, no statistically significant differences were found in %sRaw or other BP parameters between the sensitization groups (**figure 2** and **online supplements table VIS**). While in adults, %sRaw was significantly higher in adults polysensitized to 2 allergens *vs* monosensitized. In adults, no other BP parameter presented significant differences among groups of allergen sensitization (**figure 2** and **online supplements table VIIS**). After adjusting for age with ANCOVA similar findings were obtained (data not shown).

Discussion

In this study, we observed that several spirometry parameters, including %FEV1, %FVC, FEV1/FVC, %MMEF, and %PEF were significantly higher in the non-sensitized group than in at least one of the other groups (mono or polysensitized). While the % changes in FEV1 and MMEF were significantly lower in the non-sensitized group than in those polysensitized to 2 or ≥ 3 allergen groups; the % change in FEV1 was also lower in non-sensitized than in monosensitized. Regarding BP, the number of allergen sensitizations was significantly associated with %sRaw in adults, being higher in individuals polysensitized to 2

Figure 2 *- Mean with 95% confidence interval for body plethysmography, including % predicted pre-BD (panel a) and % change with BD (panel b) stratified by age group (1. All; 2. < 18 years; 3. ≥ 18 years).*



*p < 0.05; **p < 0.001.

or ≥ 3 allergen groups *vs* non-sensitized, and in those polysensitized to 2 allergens *vs* monosensitized.

This study is one of the first studies assessing the association between lung function parameters and the number of allergen sensitizations and demonstrating that individuals with allergen sensitization have worse lung function irrespective of the presence of an asthma diagnosis. In children, several previous studies have shown associations between the presence of allergic sensitization and decreased pulmonary function and nasal patency, and increased asthma morbidity (12, 20-23). In adults, allergen sensitization was related to a poorer lung function, but only in individuals with asthma (24), which differs from our findings.

Some studies reported that polysensitization is significantly associated with a poor quality of life in patients with allergic rhinitis (25) and intermittent asthma (26), with higher symptom scores for dyspnoea, wheezing, and cough (27), and with the presence of multimorbidity (7, 28, 29). Moreover, Ciprandi *et al*. have shown that impaired lung function occurs in polysensitized patients with allergic rhinitis (30); however, this study only reported on polysensitized individuals and did not clearly assess the relationship between the number of sensitizations and lung function. We could only find one study, by Nagarajan *et al*. (14), specifically examining the associations between the number of aeroallergens sensitizations and lung function parameters. In this study, in patients with highly allergic asthma (≥ 4 allergens), FEV1/FVC was significantly lower than in the group sensitized to < 4 allergens. Nevertheless, these results are not completely clear as the reported 95% confidence interval is not in agreement with a significant association (14).Therefore, the available evidence on the associations between allergen sensitizations, polysensitization with multimorbidity, and increased disease severity are globally aligned with the statistically significant associations we observed between the presence of allergic sensitization and worse lung function parameters. However, compared to monosensitized patients, we could not clearly demonstrate that spirometry or BP parameters are lower in individuals with a higher number of allergen sensitizations.

Lung function allows an objective assessment of the effect of allergic disease in airways function. With a more pronounced allergic drive, leading to sensitization to more allergens, we could expect a higher impact on lung function and lower spirometric values, as suggested by Nagarajan *et al*. (14). Nevertheless, although theoretically sound, the available evidence, including this study, does not consistently support such an association. Moreover, although we found some statistically significant associations, we could not assess the clinical relevance of the differences between groups. In fact most differences were small, of only a few percent points, in values within the normal range (*e.g.*, %FEV1 of 106% in non-sensitized *vs* 101% in polysensitized to ≥ 3 allergen groups, $p < 0.001$, corresponding to a 5% difference), which might not translate into clinical differences. Additionally, in this study, we could not account for several factors that might be relevant in the association between LFT parameters and the number of allergen sensitizations, such as ongoing inhaled medication, the relevance of the sensitizations and recent exposure to triggers, treatment with allergen immunotherapy and even the possibility of an exacerbation at the time of LFT. Also, physician diagnosis or clinical indication for LFT were not part of our anonymized database and could not be included in the analysis. Nevertheless, considering the specific setting where this study was held (an Allergy Clinic), where LFT are usually performed to patients with respiratory symptoms in the context of suspected or confirmed allergic diseases, and that only 3% of the included LFT were requested by physicians from other medical specialties (data not shown), we estimate that over 95% of the LFT were performed in patients with asthma and/or rhinitis (to assess the presence of asthma). Furthermore, this study is limited by its retrospective design and the specific setting where it was held, that limits the generalizability of our results to other clinical contexts.

Future studies are needed to assess additional clinical parameters and the impact of possible confounding variables.

Despite these limitations, this is one of the first studies showing an association between lung function parameters and the number of allergen sensitizations. The published literature is limited, and only a few studies discussed this topic. Furthermore, we analysed all the spirometry parameters and not only the main variables. Importantly, we included BP parameters that, to our knowledge, were not previously assessed in the published study that reported on the relationship between lung function and the number of allergen sensitizations.

Conclusions

In conclusion, in this retrospective study the presence of allergen sensitizations was significantly associated with worse lung function and increased bronchodilator response in spirometry and increased specific resistance in body plethysmography.

Conflict of interests

João A. Fonseca reports research agreements with AstraZeneca and Mundipharma and fees for speaking during symposia and other meetings or occasions from AstraZeneca, Mundipharma, and Viatris outside the scope of this work. Other authors declare that they have no conflict of interests.

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***Table IS** - Lung function variables that were assessed in this study.*

Spirometry	Body plethysmography
% predicted pre-BD and % change with BD - Forced expiratory volume in the first second (FEV1) - Forced vital capacity (FVC) - Maximal mid-expiration flow (MMEF75-25) - Peak expiratory flow (PEF)	**% predicted pre-BD and % change with BD** - Specific airway resistance (sRaw) - Total lung capacity (TLC) - Residual volume (RV) - Intra-thoracic gas volume (ITGV) - RV/TLC
Pre-BD - FEV1/FVC	

BD: Bronchodilator test.

***Table IIS** - Description of spirometric variables and comparison according to the number of aeroallergens sensitizations (whole sample).*

	nº sensitizations																
	Total (n =1 293)		0 (n = 402)		1 (n = 320)		2 (n = 241)		≥ 3 (n = 330)		P-value						
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Global	0 *vs* 1	0 *vs* 2	0 *vs* ≥ 3	1 *vs* 2	1 *vs* ≥ 3	2 *vs* ≥ 3
Spirometry																	
%FVC	103	13	105	14	102	13	102	13	103	12	**< 0.001**	**< 0.001**	**0.007**	0.072	1.000	0.838	1.000
FVC, % change	0.5	4.6	0.1	4.7	0.8	4.9	0.8	4.4	0.7	4.3	0.102	0.231	0.292	0.457	1.000	1.000	1.000
%FEV1	103	14	106	14	102	15	103	15	101	13	**< 0.001**	**< 0.001**	**0.007**	**< 0.001**	1.000	1.000	1.000
FEV1, % change	4.2	6.3	2.9	5.8	4.6	6.9	4.9	6.3	4.9	6.2	**< 0.001**	**0.002**	**0.001**	**< 0.001**	1.000	1.000	1.000
FEV/FVC	85	8	86	7	85	9	85	8	83	7	**< 0.001**	0.155	1.000	**< 0.001**	1.000	0.200	**0.025**
%MMEF	98	308	93	25	88	26	91	28	86	26	**0.003**	**0.043**	1.000	**0.005**	0.835	1.000	0.255
MMEF, % change	17.2	19.4	14.1	18.7	17.3	20.1	19.4	19.2	19.5	19.3	**0.001**	0.158	**0.005**	**0.001**	1.000	1.000	1.000
%PEF	103	18	105	18	100	18	103	18	103	17	**0.014**	**0.007**	1.000	0.684	0.624	0.632	1.000
PEF, % change	2.3	8.8	1.5	8.5	2.6	9.7	3.0	8.8	2.6	8.1	0.165	0.770	0.257	0.640	1.000	1.000	1.000

SD: standard deviation; FVC: forced vital capacity; FEV1: forced expiratory volume in the first second; MMEF: maximal mid-expiratory flow; PEF: peak expiratory flow; n: number; %: percent predicted.

***Table IIIS** - Description of spirometric variables and comparison according to the number of aeroallergens sensitizations in children.*

	nº sensitizations																
	Total (n = 595)		0 (n = 150)		1 (n = 177)		2 (n = 128)		≥ 3 (n = 140)		P-value						
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Global	0 *vs* 1	0 *vs* 2	0 *vs* ≥ 3	1 *vs* 2	1 *vs* ≥ 3	2 *vs* ≥ 3
Spirometry																	
%FVC	98.8	11.3	99.7	11.1	98.0	11.5	98.4	11.5	99.1	11.3	0.578	1.000	1.000	1.000	1.000	1.000	1.000
FVC, % change	0.6	4.9	0.3	5.2	0.6	5.1	1.2	4.8	0.5	4.5	0.554	1.000	0.984	1.000	1.000	1.000	1.000
%FEV1	102.8	14.2	105.9	13.6	101.8	14.7	102.3	14.7	101.3	13.3	**0.021**	0.052	0.206	**0.037**	1.000	1.000	1.000
FEV1, % change	4.2	6.3	2.6	6.0	4.6	4.1	4.9	6.6	4.5	6.3	**0.020**	0.216	**0.025**	0.095	1.000	1.000	1.000
FEV/FVC	87.8	7.8	90.3	6.1	87.0	9.4	87.9	7.5	86.0	6.9	**< 0.001**	**0.001**	0.068	**< 0.001**	1.000	1.000	0.249
%MMEF	112.5	452.7	98.7	23.1	91.3	26.1	94.8	27.4	91.3	25.5	**0.035**	0.059	1.000	0.086	1.000	1.000	1.000
MMEF, % change	17.5	21.0	13.4	19.7	17.8	22.9	20.8	21.1	18.7	19.4	**0.027**	0.348	**0.024**	0.196	1.000	1.000	1.000
%PEF	97.6	17.3	96.4	16.4	96.8	17.6	99.1	18.1	98.7	17.2	0.448	1.000	1.000	1.000	1.000	1.000	1.000
PEF, % change	2.7	10.1	1.6	10.5	2.7	10.5	3.7	10.0	3.1	9.3	0.358	1.000	0.524	1.000	1.000	1.000	1.000

SD: standard deviation; FVC: forced vital capacity; FEV1: forced expiratory volume in the first second; MMEF: maximal mid-expiratory flow; PEF: peak expiratory flow; n: number; %: percent predicted.

Table IVS - *Description of spirometric variables and comparison according to the number of aeroallergens sensitizations in adults.*

	nº sensitizations																
	Total (n = 698)		**0** (n = 252)		**1** (n = 143)		**2** (n = 113)		**≥ 3** (n = 190)		**P-value**						
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Global**	**0 *vs* 1**	**0 *vs* 2**	**0 *vs* ≥ 3**	**1 *vs* 2**	**1 *vs* ≥ 3**	**2 *vs* ≥ 3**
Spirometry																	
%FVC	106.9	13.2	108.8	13.9	105.7	13.2	106.1	12.9	105.9	12.2	**0.045**	0.154	0.400	0.116	1.000	1.000	1.000
FVC, % change	0.5	4.3	- 0.1	4.3	1.1	4.7	0.5	3.9	0.8	4.1	**0.044**	0.061	1.000	0.202	1.000	1.000	1.000
%FEV1	103.7	14.4	106.8	14.3	101.9	15.6	103.1	14.7	101.3	12.6	**< 0.001**	**0.007**	0.139	**< 0.001**	1.000	1.000	1.000
FEV1, % change	4.4	6.0	3.1	5.6	5.2	6.5	4.9	6.0	5.2	6.1	**0.001**	**0.007**	0.065	**0.002**	1.000	1.000	1.000
FEV/FVC	82.5	7.3	83.6	6.9	81.9	7.7	82.4	7.9	81.5	7.1	**0.019**	0.190	0.920	**0.017**	1.000	1.000	1.000
%MMEF	85.9	26.0	89.5	25.9	83.2	25.2	86.5	27.3	82.9	25.5	**0.029**	0.122	1.000	0.050	1.000	1.000	1.000
MMEF, % change	17.0	17.9	14.5	18.1	16.7	16.0	18.0	16.9	20.0	19.2	**0.016**	1.000	0.544	**0.010**	1.000	0.619	1.000
%PEF	106.8	17.0	109.4	17.1	104.5	17.5	106.7	17.0	105.2	16.0	**0.016**	**0.032**	0.990	0.062	1.000	1.000	1.000
PEF, % change	2.0	7.4	1.5	7.0	2.3	8.5	2.3	7.3	2.3	7.2	0.657	1.000	1.000	1.000	1.000	1.000	1.000

SD: standard deviation; FVC: forced vital capacity; FEV1: forced expiratory volume in the first second; MMEF: maximal mid-expiratory flow; PEF: peak expiratory flow; n: number; %: percent predicted.

Table VS - *Description of body plethysmography variables and comparison according to the number of aeroallergens sensitizations (whole sample).*

	nº sensitizations																
	Total (n = 287)		**0** (n = 85)		**1** (n = 66)		**2** (n = 62)		**≥ 3** (n = 74)		**P-value**						
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Global**	**0 *vs* 1**	**0 *vs* 2**	**0 *vs* ≥ 3**	**1 *vs* 2**	**1 *vs* ≥ 3**	**2 *vs* ≥ 3**
Body plethysmography																	
%sRaw	142	71	122	66	131	64	168	68	155	78	**< 0.001**	1.000	**0.001**	**0.020**	**0.018**	0.257	1.000
sRaw, % change	- 24.2	17.0	- 23.3	17.6	- 22.1	14.5	- 26.4	19.4	- 25.2	16.3	0.557	1.000	1.000	1.000	1.000	1.000	1.000
%ITGV	114	19	111	18	116	23	116	19	115	18	0.400	1.000	0.817	1.000	1.000	1.000	1.000
ITGV, % change	- 4.1	10.1	- 3.0	7.7	- 3.4	8.7	- 6.3	11.8	- 3.9	11.7	0.335	1.000	0.494	1.000	0.923	1.000	1.000
%RV	122	22	12	20	120	23	126	27	121	16	0.314	1.000	0.669	1.000	0.538	1.000	1.000
RV, % change	- 5.3	10.0	- 4.8	8.7	- 4.3	9.6	- 6.6	11.6	- 5.7	10.2	0.633	1.000	1.000	1.000	1.000	1.000	1.000
%TLC	107	11	109	13	107	12	107	9	107	11	0.663	1.000	1.000	1.000	1.000	1.000	1.000
TLC, % change	- 1.2	4.0	- 1.0	3.2	- 1.0	4.5	- 1.3	4.5	- 1.5	3.8	0.868	1.000	1.000	1.000	1.000	1.000	1.000
%RV/TLC	111	19	108	15	110	20	116	23	111	15	0.095	1.000	0.086	1.000	0.408	1.000	0.675
RV/TLC, % change	- 4.3	9.1	- 4.0	7.7	- 3.2	9.4	- 5.5	11.0	- 4.4	8.6	0.640	1.000	1.000	1.000	1.000	1.000	1.000

SD: standard deviation; sRaw: specific airway resistance; ITGV: intra-thoracic gas volume; RV: residual volume; TLC: total lung capacity; n: number; %: percent predicted.

Table VIS - *Description of body plethysmography variables and comparison according to the number of aeroallergens sensitizations in children.*

	nº sensitizations																
	Total (n = 89)		**0** (n = 11)		**1** (n = 19)		**2** (n = 30)		**≥ 3** (n = 29)			**p value**					
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Global**	**0 *vs* 1**	**0 *vs* 2**	**0 *vs* ≥ 3**	**1 *vs* 2**	**1 *vs* ≥ 3**	**2 *vs* ≥ 3**
Body plethysmography																	
%sRaw	208.3	60.7	203.8	61.1	201.2	60.3	200.3	54.4	222.9	67.2	0.476	1.000	1.000	1.000	1.000	1.000	0.939
sRaw, % change	- 22.5	15.0	- 22.1	16.1	- 23.2	14.6	- 20.3	17.1	- 24.3	12.9	0.802	1.000	1.000	1.000	1.000	1.000	1.000
%ITGV	118.0	19.4	105.9	21.4	123.3	25.0	120.8	16.4	116.4	16.1	0.089	0.112	0.170	0.724	1.000	1.000	1.000
ITGV, % change	- 5.8	13.2	- 2.7	11.6	- 5.4	11.4	- 8.1	12.3	- 5.0	15.9	0.688	1.000	1.000	1.000	1.000	1.000	1.000
%RV	126.2	23.7	117.8	20.2	129.7	30.8	130.7	26.2	122.4	15.3	0.310	1.000	0.747	1.000	1.000	1.000	1.000
RV, % change	- 7.6	10.9	- 5.4	8.4	- 6.8	11.6	- 9.9	11.0	- 6.7	11.3	0.592	1.000	1.000	1.000	1.000	1.000	1.000
%TLC	105.3	9.9	101.0	11.8	106.8	12.5	106.3	8.3	105.1	8.9	0.430	0.788	0.797	1.000	1.000	1.000	1.000
TLC, % change	- 2.4	5.0	- 1.6	3.6	- 2.7	5.8	- 2.8	5.6	- 2.2	4.4	0.916	1.000	1.000	1.000	1.000	1.000	1.000
%RV/TLC	118.3	21.4	114.1	13.6	120.7	26.1	121.9	24.9	114.5	16.1	0.497	1.000	1.000	1.000	1.000	1.000	1.000
RV/TLC, % change	- 5.3	10.6	- 3.9	7.0	- 3.9	12.8	- 7.2	11.6	- 4.8	9.3	0.727	1.000	1.000	1.000	1.000	1.000	1.000

SD: standard deviation; sRaw: specific airway resistance; ITGV: intra-thoracic gas volume; RV: residual volume; TLC: total lung capacity; n: number; %: percent predicted.

Table VIIS - *Description of body plethysmography variables and comparison according to the number of aeroallergens sensitizations in adults.*

	nº sensitizations																
	Total (n = 198)		**0** (n = 74)		**1** (n = 47)		**2** (n = 32)		**≥ 3** (n = 45)			**P-value**					
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Global**	**0 *vs* 1**	**0 *vs* 2**	**0 *vs* ≥ 3**	**1 *vs* 2**	**1 *vs* ≥ 3**	**2 *vs* ≥ 3**
Body plethysmography																	
%sRaw	112.8	53.7	110.1	58.1	102.5	39.2	136.8	65.4	110.9	46.0	**0.038**	1.000	0.110	1.000	**0.031**	1.000	0.213
sRaw, % change	- 25.1	18.0	- 23.6	18.0	- 21.5	14.7	- 32.7	20.0	- 25.8	18.4	0.092	1.000	0.206	1.000	0.099	1.000	0.768
%ITGV	112.5	19.2	112.1	17.6	112.7	21.3	111.7	20.5	113.6	19.1	0.969	1.000	1.000	1.000	1.000	1.000	1.000
ITGV, % change	- 3.2	8.0	- 3.0	6.8	- 2.5	7.1	- 4.4	11.1	- 3.3	8.1	0.839	1.000	1.000	1.000	1.000	1.000	1.000
%RV	119.6	20.2	120.6	20.6	115.6	17.4	121.6	26.8	120.5	16.6	0.489	1.000	1.000	1.000	1.000	1.000	1.000
RV, % change	- 4.2	9.3	- 4.7	8.8	- 3.1	8.3	- 3.2	11.5	- 5.0	9.6	0.753	1.000	1.000	1.000	1.000	1.000	1.000
%TLC	108.4	11.8	109.8	13.2	107.7	11.5	106.8	9.6	107.9	11.5	0.607	1.000	1.000	1.000	1.000	1.000	1.000
TLC, % change	- 0.5	3.2	- 0.8	3.2	- 0.2	3.5	0.4	2.2	- 1.0	3.3	0.292	1.000	0.748	1.000	1.000	1.000	0.563
%RV/TLC	107.3	15.9	107.1	15.1	105.4	15.9	109.7	20.4	108.0	13.9	0.685	1.000	1.000	1.000	1.000	1.000	1.000
RV/TLC, % change	- 3.7	8.2	- 4.0	7.9	- 2.9	7.4	- 3.8	10.2	- 4.1	8.1	0.923	1.000	1.000	1.000	1.000	1.000	1.000

SD: standard deviation; sRaw: specific airway resistance; ITGV: intra-thoracic gas volume; RV: residual volume; TLC: total lung capacity; n: number; %: percent predicted.

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Prevalence of Undiagnosed Common Variable Immunodeficiency in adult patients with Immune Thrombocytopenic Purpura. A single center experience

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Key words

Common variable immunodeficiency; immune thrombocytopenic purpura; primary immunodeficiency; thrombocytopenia; autoimmune anemia.

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To the Editor,

common variable immunodeficiency (CVID) is a primary immunodeficiency syndrome resulting in recurrent infections along with increased susceptibility to malignancy and autoimmunity (1). Up to 20-40% of patients with CVID develop autoimmune hematological disorders, with immune thrombocytopenic purpura (ITP) being the most frequently reported manifestation (2). The mechanism of autoimmunity in CVID is incompletely understood but is thought to be secondary to the lack of switched memory B cells, failure of removal of self-reactive B cells as well as abnormalities in B and T cell interaction (3). Although cytopenias such as autoimmune hemolytic anemia (AIHA) and ITP can precede the diagnosis of CVID (2, 4-6), it is unknown how many patients presenting with these cytopenias have undiagnosed CVID, a condition where there is commonly a delay in diagnosis, particularly in adults (7). To help answer this, we performed a retrospective cohort study with the primary objective of identifying the prevalence of CVID in patients hospitalized with ITP.

With the approval of Rochester Regional Health's Institutional Review Board (IRB), we used the International Classification of Diseases Tenth Revision (ICD-10) codes to identify patients admitted at Rochester General Hospital between January 1st, 2016 to December 31st, 2018 requiring treatment for acute ITP, or community-acquired pneumonia (CAP) while having previously documented history of ITP. Patients < 18 years of age or those with pregnancy-related or drug-induced ITP were excluded. For eligible patients, we reviewed the electronic medical record (EMR) from the time of

index hospitalization to November 2020 to determine whether a diagnosis of CVID was later established. The diagnosis of confirmed CVID was defined as a marked decrease total in IgG (> 2 standard deviation below normal age-adjusted level) and IgA titers with or without low total IgM level in the absence of secondary causes of hypogammaglobulinemia (*e.g.*, the use of anti-CD20 therapies or hematologic malignancies, *etc.*) and having a documented poor antibody response to at least one vaccine (*i.e.*, the absence of protective levels of antigen-specific IgG titers four weeks post vaccination) (8, 9). Probable CVID was defined as total IgG titer > 2 SD below normal age-adjusted level and IgA titers with or without low total IgM levels in the absence of secondary causes of hypogammaglobulinemia (8). All patients with admitted with acute ITP had a > 50% decrease from their baseline platelet count and were identified to have ITP by a board-certified hematologist. All patients admitted with CAP who had a prior history of ITP also had documentation in the chart of ITP confirmed by a board-certified hematologist.

Forty-nine unique patient admissions met the inclusion criteria within the three-year study period (**figure 1**). Eight of the 49 patients (16%) had CAP with an established history of ITP. All patients admitted with acute ITP (n = 41) had at least moderate ITP defined as platelet count between 50,000 to 100,000 μ/l. Thirty-seven of these patients had severe ITP defined as a platelet count of less than 10,000 μ/l. Patients with a previous history of ITP who were admitted with CAP either had moderate thrombocytopenia or mild thrombocytopenia defined as platelet count > 50,000 μ/l but less than 150,000 μ/l. Half of these patients (n = 4) were on low dose oral prednisone daily for chronic ITP. The median age of the cohort was 68 (IQR 57-82) years of age. Twenty-four patients (49%) were females. Thirty patients (61%) were treated with intravenous immunoglobulin (IVIG) therapy. Four patients (8%) had their immunoglobulin (Ig) levels checked on index hospitalization, while 11 patients (22%) had their Ig levels checked since the index hospitalization to November 2020. Three patients (6%) were identified to have laboratory evaluation concerning for CVID (**table I**).

The first patient was a 35-year-old female with a history of ITP diagnosed at age 29 with a nadir platelet count of < 10,000/μL requiring IVIG treatment previously. She had two episodes of rhinosinusitis within the past year requiring outpatient antibiotic therapy before being admitted for sepsis from CAP requiring oxygen support. IgG at the time of admission was less than 400 mg/dl and she was referred to Allergy/Immunology on discharge for a complete immune evaluation, where she was diagnosed with confirmed CVID at age 35 due to low IgG, low IgM, and poor response to vaccines, particularly Pneumovax (PPV23).

The second patient was a 37-year-old female who was admitted with uncomplicated pyelonephritis and thrombocytopenia due to acute ITP with a platelet count of 57,000 μL. She was hospitalized twice in the previous two years for CAP, prompting immune screening that revealed undetectable levels of IgG, IgA, and IgM. She did not respond to PPV23 was therefore diagnosed with confirmed CVID two months later and started therapy with intravenous immunoglobulin (IVIG).

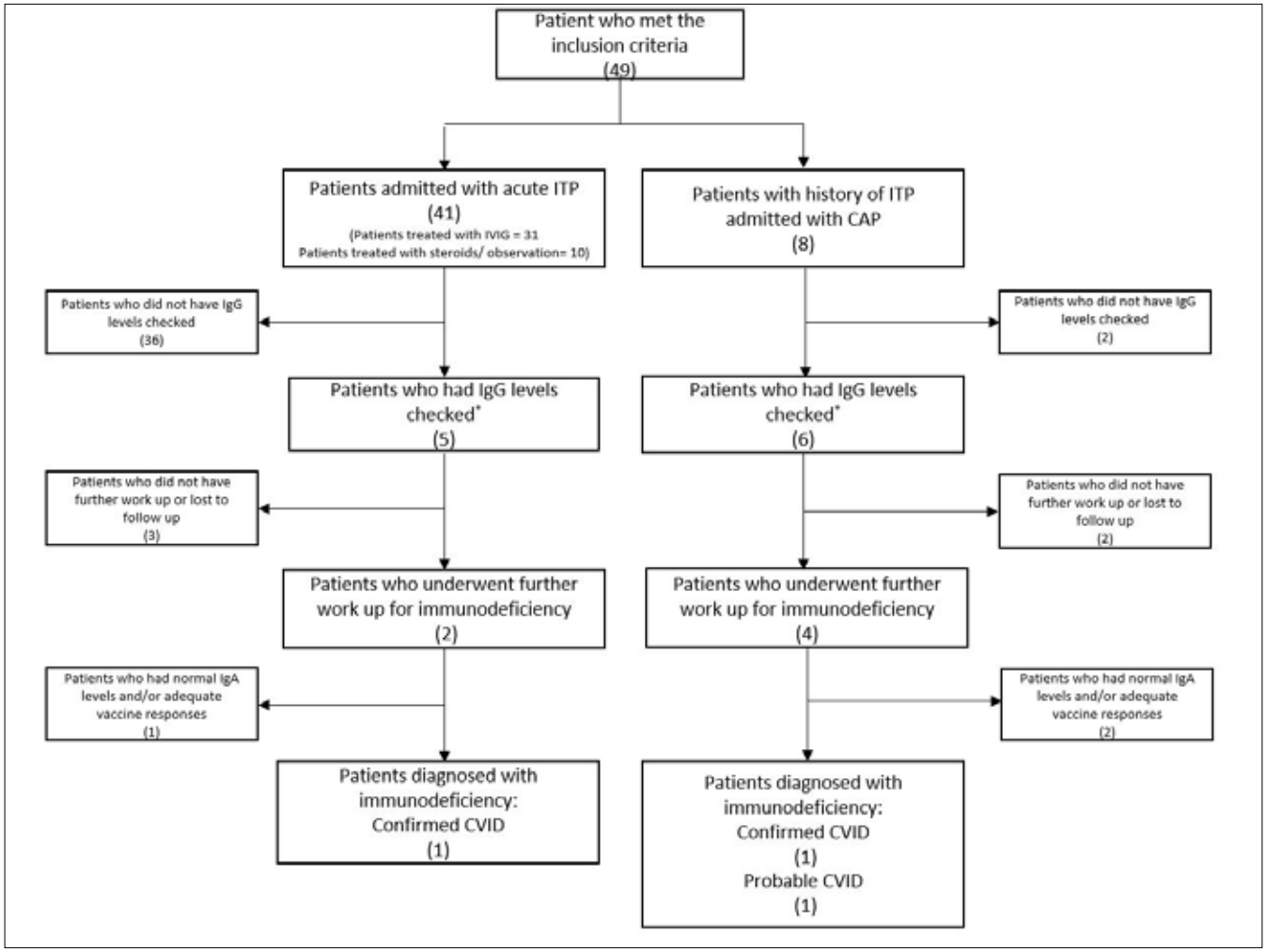
The third patient was a 53-year-old female with a history of ITP diagnosed at age 50 and end-stage renal disease on peritoneal dialysis who was admitted hypoxic respiratory failure thought to due to volume overload and superimposed CAP. The platelet count on admission was 18,000/μL. Her hospital stay was complicated by peritonitis resulting in severe septic shock secondary to peritoneal dialysis catheter placement. Immune evaluation revealed significantly low IgG, IgM, and IgA titers highly suggestive of probable CVID, but a complete evaluation was not completed because the family opted for comfort care.

Table I *- Summary of patients diagnosed with confirmed and probable common variable immunodeficiency syndrome.*

Patient	Age/Gender	Reason for hospitalization	Platelet count/μl*	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)	Response to vaccine**	Diagnosis
1	35/F	CAP	4000	400	< 5	< 5	No	Confirmed CVID
2	37/F	Acute ITP, uncomplicated pyelonephritis	57000	< 70	< 8	< 18	No	Confirmed CVID
3	53/F	Uncomplicated CAP, volume overload from missed hemodialysis session	2000	314	30	60	Not performed	Probable CVID

F: female; CAP: Community acquired pneumonia; CVID: common variable immunodeficiency syndrome. *Platelet count nadir at the time of diagnosis of immune thrombocytopenic purpura. **An adequate response to *Streptococcus pneumoniae*; PPV23 vaccine was defined as a two-fold increase from baseline if pre-vaccination specific IgG were ≥ 1.3 mcg/ml, or if titers increased by four-fold from baseline if pre-vaccination specific IgG were < 1.3 mcg/ml, for > 70% of the pneumococcal serotypes (9). Reference ranges: IgG: 700-1600 mg/dl, IgA: 70-400 mg/dl, IgM: 50-300 mg/dl.

Figure 1 *- Description of the study population.*



*Includes patients who had their IgG level checked on index hospital and those who had it checked between index hospitalization to November, 2020.

Our results mirror previous studies that have described a subset of patients with CVID who present with autoimmune, rather than infectious complications. In a retrospective chart review of 326 patients with CVID by Wang *et al.*, fifteen patients (4.6%) had ITP. Of those 15 ITP patients, nine patients had an episode of ITP before the diagnosis of CVID (4). Similarly, in a prospective study of 224 patients with CVID, 5.6% had a concurrent diagnosis of ITP. (5) In a small study of 21 CVID patients, 62% of patients had an episode of ITP within six months before being diagnosed with CVID (6). Early recognition of CVID has important therapeutic implications, especially in patients who require long-term immunosuppressive therapies such as danazol, mycophenolate, and rituximab, all of which may increase the risk of life treating infections in patients with unrecognized CVID (6, 10, 11). Furthermore, it is also important to evaluate for CVID in cases of ITP requiring splenectomy, as the routine administration of pre-operative vaccinations would likely not lead to an adequate immune response in patients with CVID (11).

Our study has important limitations, most notably a small sample size and short follow-up period. We also screened for eligible patients through inpatient hospitalization records and therefore did not include a considerable number of patients with ITP that were managed as outpatients. Despite these limitations, we feel our results address an unmet need for screening for CVID in adult patients admitted with ITP.

In conclusion, physicians should be aware of ITP as an autoimmune manifestation that may precede the classical infectious complications of CVID. Larger studies with longer follow up are needed to

determine the prevalence of occult CVID among patients with ITP. Nevertheless, studies to date suggest that clinicians should screen for CVID in patients with ITP, particularly those with a history of CAP requiring hospitalization, since early detection of CVID may lead to the prevention of recurrent infections and other complications.

Conflict of interests

The authors declare that they have no conflict of interests.

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Quality of life in severe asthmatic patients treated with benralizumab

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Key words

Quality of life; severe asthma; benralizumab; control correlation; lung function correlation.

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To the Editor,

severe asthma has a great impact on the health related quality of life (HRQoL) of patients and their families (1). Thus, an appropriate asthma control is expected to improve HRQoL. Biologics, primarily monoclonal antibodies, have been developed to target specific pathways and important molecules in the pathogenesis of asthma, and their use has shown some promising effects on the HRQoL of severe asthmatic patients (2).

Benralizumab treatment decreases exacerbations and improves lung function in patients with severe, uncontrolled eosinophilic asthma (3), and we have reported in a real-life study of 10 patients with severe asthma (4), that benralizumab decreases the number of exacerbations improving disease control.

The aim of this work is to study the effect of benralizumab in the HRQoL of patients with severe asthma, its different domains, and the relationship of the improvement in HRQoL with the changes in other variables such as asthma control, exacerbations, and lung function. The study was conducted in routine clinical practice, in accordance with the ethical standards established in the Declaration of Helsinki of 1946, and informed consent was obtained from all participants before enrolment in the study.

We have measured epidemiological variables, eosinophilia, HRQoL, number of exacerbations, asthma control (Asthma Control Test questionnaire), and lung function. We have assessed HRQoL by applying the mini AQLQ (Asthma Quality of Life Questionnaire); this questionnaire covers four health domains: symptoms, activity limitation, emotional function, and discomfort due to environmental stimuli. The nonparametric Wilcoxon signed-rank test has been used for the statistical analysis and the results are described by median and interquartile range (IQR). We have correlated the changes in quality of life data with the changes in other variables such as lung function, asthma control and exacerbations using the Spearman's rank correlation coefficient. We present data from 15 patients (9 women, 6 men) with severe eosinophilic bronchial asthma who have received treatment with benralizumab for 6 months. The mean age was 58.8 years (range 39-78), nine were never smokers and 6 ex-smokers. Most of them had overweight with an average BMI of 33.14.

Regarding background, 10 patients referred chronic rhinosinusitis, 8 nasal polyposis, and eight were atopic. About "asthma severity", nine patients were in step 5 of GEMA − Spanish Guide for Asthma Management − (treated with high-dose inhaled corticosteroids and long-term bronchodilators, in addition to antileukotrienes and anticholinergics), and other six patients were in step 6, requiring continuous oral steroids.

Referring to adverse effects, administration of benralizumab was uneventful for 14 patients, but one developed mild fever

controlled with paracetamol. This good tolerance agrees with previous reports on the safety of the drug (5).

Mean eosinophil blood count was 522 cells/μL before the drug and 48 cells/μL after it, with 9 patients presenting 0 eosinophils.

The results of the mini AQLQ are shown in the **table I**. We have obtained a statistically significant improvement in the total score: median pre-treatment 2.93 and post-treatment 5.60 ($p < 0.001$), and in the four domains: "symptoms" 3.00 and 6.20 ($p < 0.001$), "activity limitation" 3.00 and 5.75 ($p < 0.001$), "emotional function" 2.67 and 6.00 ($p = 0.001$), and "environmental stimuli" 3.33 and 5.67 ($p = 0.043$).

All the patients achieved the minimal important difference (6) of improvement in the AQLQ Total score, 14 patients in the "limitation activity" domain, 13 patients in the "symptoms" domain, 12 in the "emotional function" domain and 8 patients in the "environmental stimuli" domain.

All patients experienced clinical improvement with the treatment. The number of exacerbations decreased in all cases, and the median for the year prior to benralizumab was 3 (2.00-4.00) and 0 (0.00-0.00) (annualized rate) after the drug ($p = 0.001$).

We also obtained improvements in asthma control: median ACT pre-treatment 12 (8.00-16.00), post-treatment 21 (19.00-23.00) ($p < 0.001$) and non-significant for lung function: median FEV1 pre-treatment 1.6 L (1.14-2.06), post-treatment 1.81 L (1.19-2.93) ($p = 0.078$ NS). We have correlated the changes in the Total AQLQ score and the four domains with the changes in asthma control, lung function and exacerbations. The total AQLQ score shows significant correlation with ACT: $r_s = 0.615$ ($p = 0.015$) and FEV1: $r_s = 0.739$ ($p = 0.002$), but not with decrease of exacerbations: $r_s = -0.430$ (p 0.109 NS) (**figure 1**). Similar results have been obtained by "symptoms", which also correlates with ACT: $r_s = 0.535$ ($p = 0.040$) and FEV1: $r_s = 0.702$ ($p = 0.004$), but not with exacerbations: $r_s = -0.419$ ($p = 0.120$ NS), and "environmental stimuli" with ACT: $r_s = 0.534$ ($p = 0.040$), with FEV1: $r_s = 0.591$ ($p = 0.020$), and exacerbations: $r_s = -0.349$ ($p = 0.203$ NS).

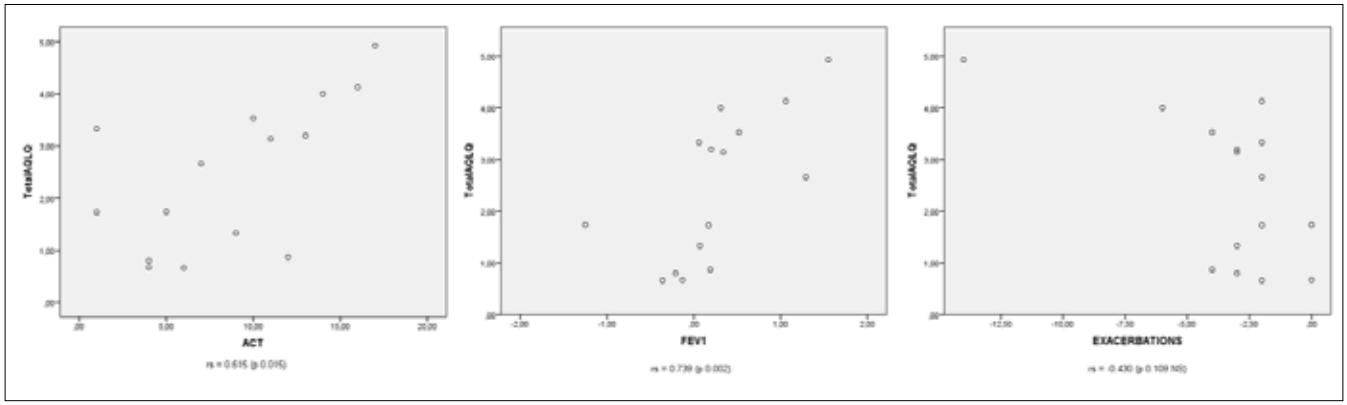
Nevertheless, the domain "activity limitation" correlates with the three variables, ACT: $r_s = 0.706$ ($p = 0.003$), FEV1: $r_s = 0.680$ ($p = 0.005$), and exacerbations $r_s = -0.518$ ($p = 0.048$). Finally, the domain "emotional function" does not correlate with any other variable: ACT: $r_s = 0.325$ ($p = 0.238$ NS), FEV1: $r_s = 0.504$ ($p = 0.055$ NS), and exacerbations $r_s = -0.286$ ($p = 0.302$ NS).

Table I *- Results of mini AQLQ questionnaire.*

AQLQ	Pre	Post	DIF	P-value
Symptoms	3.00 (2.20-4.20)	6.20 (5.80-6.80)	3.20	< 0.001
Shortness of breath (1)	3	6		
Discomfort due to coughing (4)	2	6		
Chest tightness (6)	4	7		
Sleep disturbance (8)	3	7		
Wheezes (10)	2	6		
Activity Limitation	3.00 (1.25-4.00)	5.75 (4.50-6.75)	2.75	0.001
Intense efforts (12)	1	5		
Moderate efforts (13)	3	6		
Social activities (14)	4	7		
Work activities (15)	3	6		
Emotional Function	2.67 (1.67-3.67)	6.00 (4.33-7.00)	3.33	0.001
Frustrated-irritated (3)	3	6		
Fear not having medication (5)	2	6		
Concerned about asthma (9)	2	6		
Environmental Stimuli	3.33 (2.67-6.33)	5.67 (4.00-6.67)	2.34	0.043
Discomfort due to Dust (2)	6	6		
Discomfort due to Tobacco (7)	4	7		
Discomfort due to Pollution (11)	3	6		
Total	2.93 (2.67-4.27)	5.60 (4.73-6.87)	2.67	< 0.001

Median (Interquartile range); question number in questionnaire in parenthesis.

Figure 1 *- Correlation between AQLQ and ACT, FEV1 and Exacerbations.*



We have obtained, after treatment with benralizumab, a significant improvement in the patients' HRQoL, both Total AQLQ score and the four domains "symptoms", "activity limitation", "emotional function" and "environmental stimuli". This improvement correlates with improvement in asthma control (ACT) and lung function in the case of total score, "symptoms" and "environmental stimuli", and in addition with decrease of exacerbations in "activity limitation".

In accordance with other authors, a correlation was found between improvement in HRQoL and asthma control (7) and lung function improvement. The lack of correlation of the changes in HRQoL with decrease of exacerbations (but "activity limitation") has also been reported by Enríquez-Matas *et al.* (8), and it could be due to the fact that the short period covered for the AQLQ questionnaire (two weeks) makes more difficult reflecting changes in a variable such as exacerbations. This limitation is more evident considering the long free period there is sometimes between exacerbations.

We conclude that benralizumab significantly improves HRQoL in severe asthmatic patients, globally and by domains: "symptoms", "activity limitation", "emotional function", and discomfort due to "environmental stimuli". This improvement is correlated with better asthma control and lung function.

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None.

Conflict of interests

Juan Carlos Miralles López has received lecture fees from Novartis, GSK, Astra Zeneca, Sanofi and Chiesi. The rest of authors declare that they have no conflict of interests.

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