edra | Issn 1764-1489 | Volume 55 N. 2/2023 – March 2023

European Annals of Allergy and Clinical Immunology

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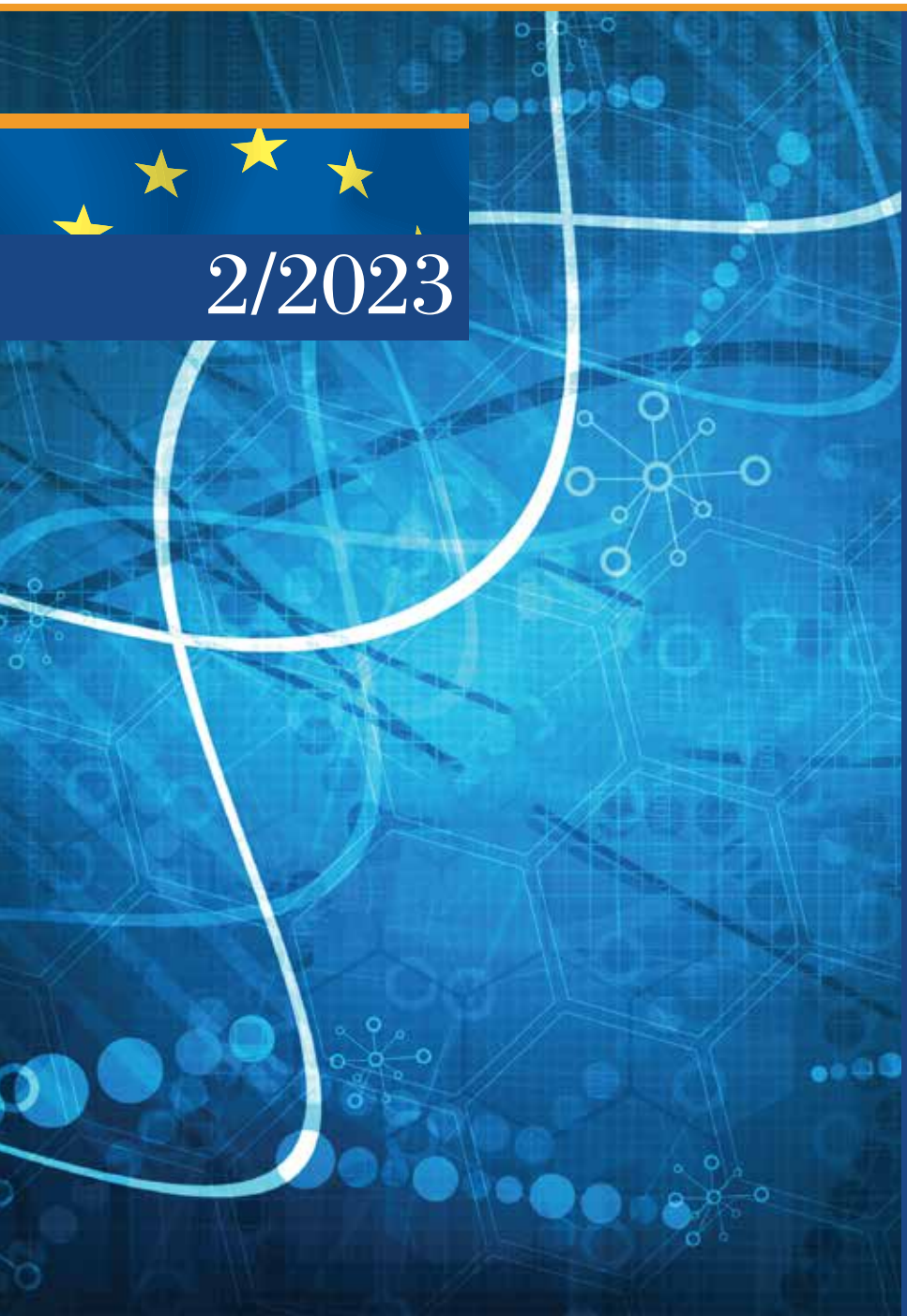

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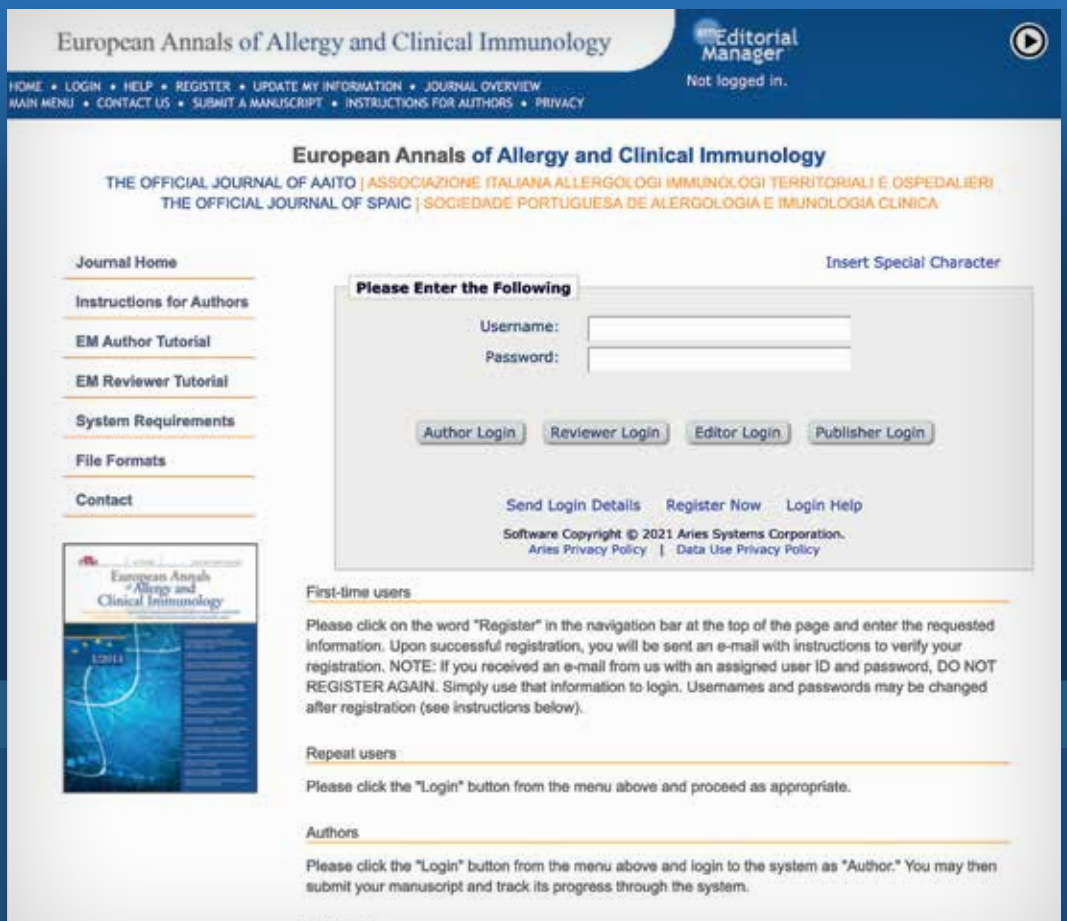

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Jessica Guenzi
j.guenzi@lswr.it
Ph. 039 (0)2-89293.926

Production Manager

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p.ficicchia@lswr.it
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Sales

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Subscription

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Ph. 0039 (0)2-88184.317
Italy subscription: 60 euro
World subscription: 85 euro

Printing

Rotomail Italia S.p.A., Strada Rivoltana (SP 14), 12/AB 20060 Vignate (MI), Italy

EDRA SpA

Via G. Spadolini, 7
20141 Milano - Italy
Tel. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
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"European Annals of Allergy and Clinical Immunology" registered at Tribunale di Milano - n. 336 on 22.10.2014

The contents of this Journal are indexed in PubMed, Scopus, Embase and Web of Science®

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SHORT REVIEW | Eur Ann Allergy Clin Immunol | Vol 55, N.2, 51-56, 2023

Gennaro Liccardi[1], Maria Beatrice Bilò[2,3], Manlio Milanese[4], Matteo Martini[2,5], Gloria Pane[6], Anna De Maio[7], Paola Rogliani[1,6]

COVID-19 lockdown, personal protective equipment, hyper-hygiene and allergy

[1]Department of Experimental Medicine, Postgraduate School of Respiratory Medicine, University of Rome Tor Vergata, Rome, Italy
[2]Department of Clinical and Molecular Sciences, Marche Polytechnic University, Ancona, Italy
[3]Allergy Unit, Department of Internal Medicine, University Hospital Ospedali Riuniti, Ancona, Italy
[4]Division of Pulmonology, S. Corona Hospital, Pietra Ligure, Savona, Italy
[5]Allergy Unit, Ospedali Riuniti Marche Nord, Fano, Italy
[6]Respiratory Medicine Unit, Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy
[7]Allergology and Drug Allergy Unit, San Paolo Hospital, Naples, Italy

Key words

Allergic rhinitis; allergy; bronchial asthma; contact dermatitis; COVID-19; face masks; hypersensitivity; hyper-hygiene; lockdown; personal protective equipment (PPE); disinfection; SARS-CoV-2.

Corresponding author
Gennaro Liccardi
Department of Experimental Medicine
Postgraduate School of Respiratory Medicine
University of Rome Tor Vergata
via Cracovia 50
00133 Rome, Italy
ORCID: 0000-0002-0433-1339
E-mail: gennaro.liccardi51@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.243

Summary

At the beginning of SARS-CoV-2 pandemic, in the absence of "targeted" therapies, the national health authorities have introduced some measures aimed at reducing the spread of infection in the community (lockdown, social distancing, personal protective equipment (PPE), personal hygiene and disinfection of living environments). All the containment measures have led to both positive and negative effects in patients with allergic diseases. We believe that further studies should be undertaken to investigate the possible correlations between SARS-CoV-2 infection and allergy, from a broader perspective. In particular, the risk factors for the development of undesirable effects should be investigated, especially in healthcare professionals forced to use PPE and sanitizing agents for a long time. However, since the COVID-19 pandemic probably will not be short-lived, the use of such protective aids will necessarily be widespread even in the general population. Therefore, further studies on the materials used for the production of PPE and sanitizing agents would be necessary to reduce their sensitizing and, in some cases, toxic potential.

Impact statement

All the SARS-CoV-2 containment measures have determined both favorable and negative effects in patients with allergic diseases.

Introduction

The role of allergic diseases and related treatments as a possible risk factor for severe SARS-CoV-2 infection has started to be investigated since the beginning of the COVID-19 pandemic, on 11th March, 2020. In patients with asthma, it has been postulated that high doses of inhaled corticosteroids might facilitate the replication of the virus in the airways, with detrimental effects especially in case of poorly controlled asthma.
The lack of specific therapies against SARS-CoV-2 pushed the Authorities to strict measures aimed at spread control: lockdown, social distancing, personal protective equipment (PPE), personal hygiene and disinfection of living environments.
This led to pros and cons for allergic patients, and the purpose of this contribution is to elucidate this topic.

Effects of SARS-CoV-2/lockdown on allergic diseases

Particular attention has been paid to the overall effect of lockdown in patients with allergic respiratory diseases (1). It is likely that the reduction of respiratory infections due to lockdown, social distancing, face masks, and hand washing had a role in the improvement of some clinical outcomes such as reduction of asthma hospitalizations (2, 3), both in adults and children (4). It has been suggested that the lockdown and the consequent changes in exposure to different kinds of pollution may have different − and sometimes opposite − effects in patients, depending on the type of sensitization, namely worsening of clinical symptoms in patients sensitized to "indoor" allergens, and improvement in those sensitized to "outdoor" allergens (5). In addition, pandemic and lockdown had an impact not only on respiratory allergies, but also on food allergy.

Respiratory allergy: effects in indoor environments

It has been hypothesized that lockdown might be a risk factor for development of allergic diseases due to the more prolonged exposure to sensitizing proteins and chemical agents present in indoor environments (6). During the lockdown, higher levels of pollutants and in particular polycyclic aromatic hydrocarbons (PAHs) were found, compared to the pre-COVID-19 period, due to the increase of domestic activities (*e.g.*, cooking, cleaning, heating) (7), and despite the decreased production of outdoor pollution and consequently of its level in indoor environments (8).
As expected, the "home confinement" due to pandemic has had negative clinical effects on patients with allergic rhinitis to dust mites. In fact, a worsening of upper airway symptoms, as well as an increase in the use of specific drugs (anti-H1 agents, nasal steroids, decongestants, *etc.*), has been documented in spring 2020, compared to spring 2019 (9). Similar results have been demonstrated by Yucel *et al.* (10) in a group of children with rhinitis and/or bronchial asthma with or without sensitization to dust mites.
In the other hand, a substantial improvement in asthma symptoms (assessed by Asthma Control Test, drug use, frequency of exacerbations, *etc.*) has been observed during the 2020 lockdown compared to the same period of the previous year. The lower frequency of any viral infections due to school closures was considered the main cause of the favorable course of asthma in children (10). However, in children with associated mite allergic rhinitis, it has been showed a significant increase in the severity of nasal and conjunctival symptoms in the period March-May 2020, compared to the same months of 2019. No significant increase in the same nasal/ocular symptoms was reported in subjects with asthma and non-allergic rhinitis (10).

Respiratory allergy: effects in outdoor environments

During the pandemic period, an improvement of allergic rhinitis has been demonstrated in patients sensitized to "outdoor" allergens, especially to pollens (5), but also regardless of the type of allergen (9, 11). Damialis *et al.* (10, 12) demonstrated, in 31 countries, that high atmospheric levels of allergenic pollens were associated with high rates of SARS-CoV-2 infections regardless of the subjects' atopic status. Just before the start of the SARS-CoV-2 pandemic, the same authors had highlighted that high allergenic pollen counts had an effect facilitating the spread of respiratory viruses as pollens were able to reduce the innate immune defenses against viruses (11, 13).
Recently, Gelardi *et al.* (14) have demonstrated a significant improvement in sino-nasal clinical outcomes (*e.g.*, nasal obstruction, postnasal discharge, thick nasal discharge, *etc.*) and a decrease of drug use in patients with seasonal allergic rhino-conjunctivitis from pollen in Italy during the lockdown, compared to the same period in 2019.

Effects on food allergy

Musallam *et al.* (15) have shown that food allergic reactions (FARs) occurred with a significant lower incidence during the lockdown period (April-May 2020) compared to the previous 3 months. There are several possible explanations for this finding. For example, primary caregivers may have been more careful in feeding their allergic children, to minimize the need of medical aid and access to the emergency room during the pandemic, or they had to eat home-made food instead of meals from restaurants due to the restrictions, which is likely to decrease the frequency of unintentional FARs (15). Moreover, Nachshon *et al.* (16) have observed, in Israel, a significant reduction in the rate of home epinephrine-treated reactions during the COVID-19 lockdown (March 15th-April 30th, 2020), in patients undergoing oral immunotherapy (OIT) for food allergy, compared with the events occurred over the same time frame from 2015 to 2019. These results suggest that potentially avoidable triggers (*e.g.*, exercise, fatigue, infections) may contribute significantly to the rate of adverse reactions during OIT (16).

The role of filtering masks in allergy

The use of face masks is particularly widespread to prevent inhalation of chemical agents in areas with high levels of pollution or in professional environments (*e.g.*, paint workers) (17, 18). Especially in Asian countries, surgical masks are used to prevent the spread of seasonal viruses such as influenza (19). In allergy practice, PPE together with nasal filters and "barrier" materials are the most common devices to avoid contact with allergens (20). PPE (associated with the use of gowns, shoe covers and protective goggles) are essential tools in the prevention of both occupational allergy in individuals who work with animals (*e.g.*, in animal housing), and passive transfer of animal allergens from work environments to private houses (21, 22).

Unsurprisingly, the massive use of PPE has led to significant inconveniences in the millions of people who have been forced to wear PPE for many hours a day (22). Difficulties in breathing, communicating and recognizing faces, dermatological issues, sweating, *etc*. represent the most common discomforts reported by patients (23).

Possible role of filtering masks in pollen allergy

Very few studies have shown the effectiveness of PPE in people with allergic rhinitis. Dror *et al*. (24) have documented that the use of professional PPE (surgical or N95) reduced the severity of symptoms of chronic allergic rhinitis (regardless of the type of allergen) in healthcare professionals. The nurses scored their allergic rhinitis symptom severity before and after wearing face masks for 1 week at work.

Godoh *et al*. (25) documented a reduced penetration of Japanese cedar pollens in eyes and nasal cavities by using face-masks and eyeglasses, but no data were collected about symptoms.

Since in Campania region (Italy), during the COVID-19 lockdown, the use of protective masks outdoors has been mandatory since April 2020 and considering that April is a peak period of pollen release of some common herbaceous species, such as *Parietaria* (26, 27), Liccardi *et al*. (28) compared, in patients with seasonal allergic rhinitis (SAR), the self-reported symptoms experienced in April 2020 (with face masks) with the ones of April 2019 (without face masks), and the correlation with time-of-use of masks, taking also into account the role of potential confounders (changes in pollen and pollution levels). Thirteen Allergy units or Centers belonging to the Italian Association of Hospital and Territorial Allergologists (AAIITO, Campania Region) participated in the study. The patients used non- standardized face masks mainly made of different washable fabrics, because of the well-known shortage of medical face masks during the first months of SARS-CoV-2 pandemic. Data showed similar and even higher environmental pollen levels in April 2020, compared to April 2019, stable values of PM2.5, PM10, slight increases of O^3, and a reduced trend of other pollutants. Based on this background, the results of the real-world study suggest that simple non-professional face masks can reduce the nasal symptoms of SAR induced by seasonal pollens, at least during seasonal pollen peaks. Certified and professional face masks (*e.g.*, N95, FFP2) are likely to be even more effective, since they can filter also the ultra-fine components of pollen grains (28).

COVID-19 PPE, hygiene and allergy

The rapid and dramatic increase in the use of PPE (face masks, gloves, gowns, shoe covers, *etc*.) and sanitizing chemicals for hands and surface cleaning has led to an increasing amount of adverse events, especially in healthcare professionals (29), but also in the general population (30). Frequent use of hand sanitizers (containing antimicrobial agents, sensitizing compounds, *etc*.) has increased the occurrence of contact dermatitis especially in healthcare professionals (31). The increase in reactive hand contact reactions was documented among surgeons and anesthesiologists by comparing the frequency of these events before and during the months of the pandemic (34).

Although hands are the most frequent target of contact dermatitis, case reports have documented significant facial contact dermatitis after prolonged use of surgical polypropylene face masks (33), probably due to formaldehyde and 2-bromo-2-nitropropane-1,3-diol (33).

Corazza *et al*. (34) have shown that surgical masks can induce even severe contact urticaria, even if the diagnostic tests were unable to highlight the sensitizing agent(s). Face masks can cause adverse events also in the ENT area.

An online survey conducted among healthcare professionals highlighted the benefits of using face masks: reduction of aerosol transmission, protection from pollution and infections, reduction of nasal crusting, prevention of risky habits like nose picking or face touching (35). However, several drawbacks of using face masks have also been reported, like fogging of eyeglasses, ear pain due to elastic band, difficulty in breathing, excessive sweating, skin marks and scarring due to pressure, *etc*. (35).

Primov-Fever *et al*. (36) reported deterioration of sinonasal quality of life in the COVID-19 pandemic period, possibly caused by mask-wearing, especially for a prolonged time, irrespective of the mask type.

Irritant rhinitis (IR) is defined as an inflammatory and/or irritative response of the nasal mucosa due to non-allergic stimuli, *e.g.*, a physical or chemical stimulus. IR has been found in 46 patients with nasal symptoms upon usage of FFP masks in private or professional environments, and this diagnosis was confirmed by the finding of polypropylene fibers in nasal lavage fluids (37).

The findings of the studies on face masks should encourage medical companies to produce more "airway-minded" PPE, considering also the high request for these devices in the future, due to COVID-19 pandemic or other possible pandemics.

It is also worth noting that the massive use of sanitizing agents (alcohol-based products) in spray formulations for the sterilization of surfaces and confined environments can induce an "ocular surface disease" in the absence of adequate protections (38). Serious undesirable effects may result by mixing different cleaning products, as this can generate hazardous fumes/gases (39). Chronic exposure to these gases can induce asthma and chronic bronchitis (40).

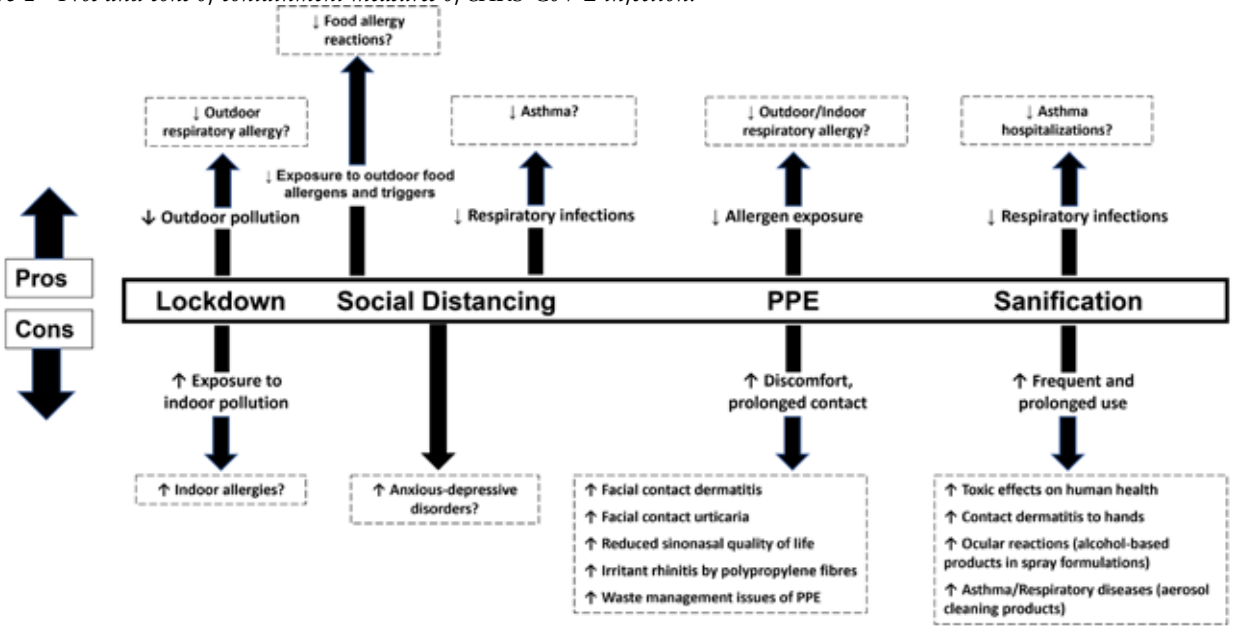
Lessons learnt from the "pandemic model"

The SARS-CoV-2 pandemic and the related lockdown can be considered a study model to evaluate the possible effects of these events in allergic patients, particularly in case of respiratory allergy. During the interruption – or massive reduction – of many human activities, there has been a drastic decrease in pollution of external environments with positive effects in patients with respiratory allergies due to the reduction of the "adjuvant" and "direct" effects on the airways. On the contrary, the compulsory indoor confinement has increased the reactivity of the airways to chemical agents and allergens of indoor environments. This "study model" confirmed the key role of pollution on airway inflammation and that, in the industrialized countries, a free-of-pollution environment does not really exist.
Furthermore, social distancing is likely to have reduced the circulation of seasonal viruses which commonly act as asthma exacerbating factors. In fact, the urban lifestyle is characterized by the frequent gathering of people both in open (*e.g.*, stadiums, public events in general, *etc.*) and confined environments (subways, theaters, cinemas, schools, *etc.*).
Another lesson learnt from the pandemic/lockdown is the usefulness of the face masks. This device, especially in the FFP2 or N95 version, is crucial in the prevention of viral SARS-CoV-2 and bacterial infections, but it has also proved good efficacy in reducing the symptoms of allergic rhinitis, being able to filter both allergens and pollutants. A more widespread use of masks by patients with respiratory allergy in the presence of high environmental levels of allergens, pollutants or micro-organisms would therefore be recommended, even after the pandemic emergency.
Concerning the hyper-hygiene state caused by the pandemic, although disinfectants and sanitizers have a key role in the prevention and control of COVID-19, important concerns must be considered about their large-scale use, including the side effects on human and animal health along with harmful impacts exerted on the environment and ecological balance (41).

Conclusions

The review of the literature shows that the containment measures adopted around the world against the COVID-19 pandemic can induce both positive and negative effects in subjects with allergic diseases (**figure 1**). We believe that further studies should be undertaken to investigate the possible correlations between SARS-CoV-2 infection and allergy, from a broader per-

Figure 1 *- Pros and cons of containment measures of SARS-CoV-2 infection.*



--------: Hypothesis drawn from the current review, to be confirmed by further data.

spective. In particular, the risk factors for the development of undesirable effects should be investigated, especially in healthcare professionals forced to use PPE and sanitizing agents for a long time. However, since the COVID-19 pandemic probably will not be short-lived, the use of such protective aids will necessarily be widespread even in the general population. It has also been suggested that improper contacts and relationships between humans and animals (particularly birds), and other conditions related to the environment, could lead to the onset of other pandemics in the future (42).
Therefore, further studies on the materials used for the production of PPE and sanitizing agents would be necessary to reduce their sensitizing and, in some cases, toxic potential.

Previous presentations

Data have been presented at AAIITO Webinar "SARS-CoV-2 e l'allergologo" – 4th June 2021.

Fundings

None.

Contributions

All authors contributed equally to this work.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgements

We thank Maria Vittoria Liccardi for technical assistance in the preparation of this manuscript.

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Eur Ann Allergy Clin Immunol Vol 55, N.2, 57-67, 2023

Bárbara Kong Cardoso[1], Laurinda Silva[2], Ana Marta Magalhães[3], Sofia Martins Farinha[1], Teresa Antunes[3], Cristina Mourato[2], Elza Tomaz[1], Filipe Inácio[1]

Primary care doctors' attitude towards drug allergy in Portugal: a questionnaire

[1]Immunoallergology Service, Hospital Center of Setúbal, Setúbal, Portugal
[2]USF Luísa Todi, ACES Arrábida, Lisbon and Tagus Valley Regional Health Administration (ARSLVT), Lisbon and Tagus Valley, Lisbon, Portugal
[3]USF Santiago, ACES Arrábida, Lisbon and Tagus Valley Regional Health Administration (ARSLVT), Lisbon and Tagus Valley, Lisbon, Portugal

Key words

Drug hypersensitivity; drug allergy; primary health care; medical education; questionnaires.

Corresponding author
Bárbara Kong Cardoso
Immunoallergology Service
Hospital Center of Setúbal
Hospital de São Bernardo
R. Camilo Castelo Branco 175
910-549 Setúbal, Portugal
ORCID: 0000-0002-0304-0126
E-mail: barbarakc@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.216

Summary

Background. *The aim of the study was to learn about perception of drug allergy by general practitioners (GP) from continental Portugal, identify difficulties and educational needs for its management.* ***Methods.*** *A total of 372 answers were obtained. A questionnaire was addressed to GPs.* ***Results.*** *The most commonly identified drugs were antibiotics for 65.3% of the GPs and skin was the most commonly affected organ for 65.8%. Drug allergy was considered as very important in clinical practice by 73.7%, but difficulties in recognizing it were stated by 70.2%. Further education in this field would be welcome by 97.8% of the doctors. The collaboration of Immunoallergology centers was considered non satisfactory by 39.8% of GPs and 45.7% of them stated that two-thirds of the suspected reactions were not investigated.* ***Conclusions.*** *These points deserve consideration in future health educational and organizational strategies.*

Impact statement

This national survey recognized drug allergy as an important problem in clinical practice. It was evident the interest in allergology trainings and in a better Immunoallergology centers response.

Introduction

World Health Organization (WHO) defines an adverse drug reaction (ADR) as a dose-independent, unpredictable, noxious, and unintended response to a drug taken at a dose normally used in humans (1).

Rawlins and Thompson in 1977 and 1981 proposed a classification of ADR, which is the most commonly used classification until today (2, 3). Two groups are considered: type A reactions (predictable, dose dependent, based on pharmacological mechanism and possible in all patients if enough dose is administered) and type B (unpredictable, not dose dependent occurring in a small group of susceptible patients). Less common types of reactions were later described: type C (chronic, dose and time dependent), type D (delayed reactions), type E (end of treatment, withdrawal reactions), and type F (unexpected failure of therapy) (4, 5). ADRs must be notified to the National Pharmacovigilance System (6).

Only 10-15% of all ADR reactions are type B being the majority type A reactions (7). Type B reactions are hypersensitivity reactions to drugs and are classified as allergic when an immunological mechanism mediated by IgE or T-cell is involved (8).

An allergic drug reaction may be diagnosed by a specific allergy workup conducted by an Immunoallergology specialist in safe and controlled conditions.

ADRs are an important public health problem, not only associated with significant morbidity and mortality but also to unnecessary costs. Several authors report a high number of hospital

admissions due to ADRs (9). However, in spite of the increase in ADRs reports in the last years, particularly of severe reactions (6), its prevalence is unknown, since there aren't epidemiological studies in most countries (7).

In the majority of studies, the distinction between an allergic and a non-allergic reaction to drugs isn't clear (10). The lack of differentiation between these two types of reactions is also noticed in clinical practice and may determine the exclusion of first line therapeutic drugs, leading to an unnecessary and possibly counterproductive attitude in non-allergic reactions.

The absence of allergology and clinical immunology areas in medical education and training programs contributes largely to the difficulty in the management of drug hypersensitivity. This gap is identified by the European Academy of Allergy and Clinical Immunology (EAACI) position paper about the allergy management in primary care. It emphasizes the need for homogenous and well-structured education programs in these areas (11). Further, an EAACI task force suggested a diagnosis and management approach of ADRs for primary care physicians, including the recognition of red flags and referral criteria (12).

Being the first line of health care providers, General Practitioners (GPs) must cope with most ADRs and their clinical decisions have a major impact on the general management of ADRs.

A study conducted in Romania by Mihaela Leru focused on the GPs clinical practice, since they are the closest doctors to patients and the main source of drug prescription. The conclusions of this study also highlighted the need for further educational programs for GPs in drug allergies and pointed out that their knowledge of this subject, as well as their collaboration with Allergologists, wasn't standardized (13).

The aim of our study was to evaluate GPs' perception of the problem of drug allergy and identify the difficulties they encounter while managing ADRs, as well as the educational needs on this subject in different areas of Portugal.

Materials and methods

Type of study and participants

We performed a cross-sectional study based on a questionnaire addressed to all GP specialists and trainees from continental Portugal. The questionnaire was available from the 1st of June 2018 to the 31st of May 2019. According to the information provided by the Central Administration for Health System (ACSS), the total number of GP interns and specialists in May 2019 was 7,931 (2,238 and 5,693, respectively).

As demanded by the Regional Health Administration (Administração Regional de Saúde - ARS) Ethics Committees, the questionnaire was sent by electronic mail to all Health Center Clusters (Agrupamentos de Centros de Saúde - ACES) in Continental Portugal, from where they were addressed to the Health Center coordinators, and finally forwarded to family doctors.

Questionnaire

An online questionnaire was created using Google Docs® software. The participation was voluntary, anonymous, unpaid and confidential. Data was used within the scope of this study and only available to researchers. The questionnaire included 23 questions: 2 questions focused on the professional experience and the geographical location; 17 questions on the perception of drug allergy epidemiology and how to manage ADR; and 4 questions addressed the educational needs in allergology (questionnaire included in **online supplements appendix 1**).

Ethical considerations

The study was approved by the Ethics Committee of ARS Norte, ARS Centro, ARS Lisboa e Vale do Tejo, ARS Alentejo and ARS Algarve, in compliance with the Helsinki Declaration.

Data processing and analysis

Data was collected and analyzed using an Excel© spreadsheet, protected with a password.

To calculate the study accuracy Krejcie & Morgan formula was applied (14). Chi-square statistic was used for testing relationships on variables (categorical).

For statistical analysis IBM SPSS Statistics for Windows, version 25 was used.

Results

Applying the Krejcie & Morgan formula to our population (7,931), the 372 completed questionnaires obtained allowed for a confidence level of 95% and 5% margin error (14). The minimum number to reach those parameters would be 363 questionnaires.

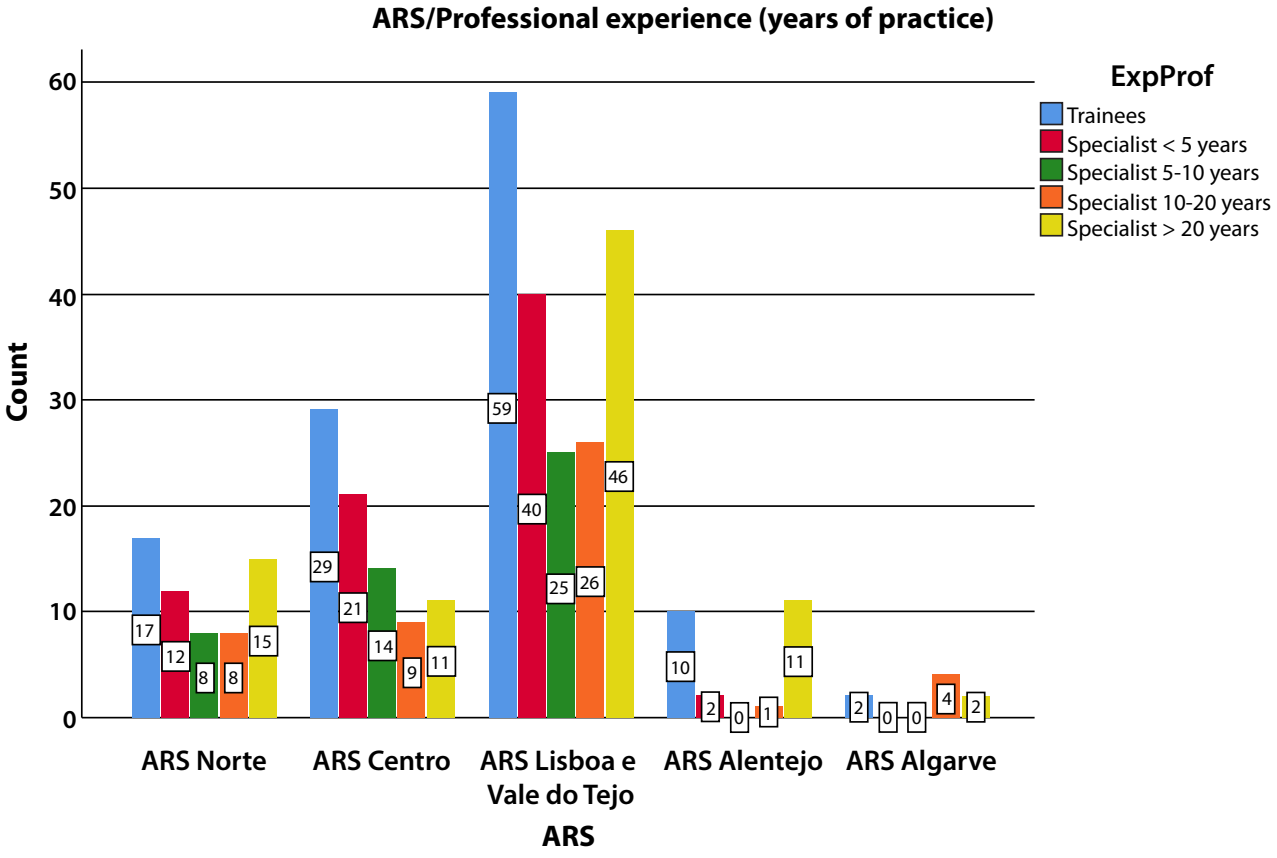
Figure 1 shows participant characterization. Out of the 372 completed questionnaires, 117 (31.5%) were filled by GP trainees and 255 (68.5%) by specialists. Sixty (16%) were received from ARS Norte, 84 (22.6%) from ARS Centro, 196 (52.7%) from ARS Lisboa e Vale do Tejo, 24 (6.5%) from ARS Alentejo and 8 (2.2%) from ARS Algarve.

Responses from all professional experience levels considered were obtained in the most represented ARS.

The GPs' perception of drug allergy is summarized in **table I**. Nearly half of the doctors described that the incidence of drug allergy is increasing. The clinicians identify antibiotics as the main cause of drug allergy (65.3%), followed by non-steroidal anti-inflammatory drugs (19%). The "patient insecurities in the future use of other medicines" and the "difficulty in finding appropriate therapies" were pointed out as the most common consequences of a drug allergy episode. Most of the clinicians were aware that drug allergies carried a risk of a fatal outcome.

Regarding the impact of drug allergy in their practice (**table II**), 73.7% of GPs considered it a very important issue and 75.5% observed it monthly or twice a year. Nearly half of them estimated

Figure 1 *- Participant characterization (n = 372) according to ARS and Professional experience.*



fewer than 30 cases of drug allergy in their patient list, 59.4% considered that this condition had some clinical impact on their therapeutic decisions. Drug allergy was stated as not having been investigated in any patient with a clinical suspicion by 27.4% of clinicians and investigated in about one third of the patients by 45.7%.

Table III provides information on drug allergy recognition and management. Skin involvement and facial edema were considered the most characteristic features of drug allergy by the majority of GPs (65.8%) and almost all chose to use an alternative drug in this situation (97.3%). A severe drug reaction, the difficulty in finding an alternative therapy and pediatric patients were the most common reasons for referrals to an allergologic study. Seventy percent recognized having difficulty in distinguishing an allergic reaction from a non-allergic reaction to a drug. Considering the levels of experience this difficulty ranged from 60.0% (more than 20 years of experience) to 77.3% (less than 5 years of experience), no statistical difference was found.

One hundred and eighty-five (49.7%) of the doctors have never notified the National Pharmacovigilance System.

The perceived delay in obtaining an Immunoallergology appointment was less than 6 months for most cases in Norte (73.3%), Centro (86.9%) and in Lisboa e Vale do Tejo (74.0%), whilst in Alentejo it was only 37.6% and in Algarve 50.0%. The difference between the highest and lowest value is significantly different (χ^2 = 22.29, P-value < 0.01).

As for global GP satisfaction levels it ranged from 72.6% in Centro to 29.2% in Alentejo (**table IV**), which was statistically different (χ^2 = 15.11, P-value < 0.01). The doctor's satisfaction is related to the delay of Immunoallergology response: for a delay less than 3 months 91.2% of doctors considered it satisfactory, while for a delay longer than 3 months only 40.2% expressed satisfaction. The difference between satisfaction levels is significant (χ^2 = 97.1, P-value < 0.001).

Globally, 39.8% had no drug allergy training at all and almost all (97.8%) were interested in getting further education in this area, preferably through clinical training initiatives taking place in Health Centers (**table V**).

The rate of training in drug allergy ranged from 32.0% to 45.3% across all levels of professional experience, which wasn't significantly different.

Table I *- Perception of drug allergy (n = 372).*

	n	%
How do you describe the incidence of drug allergy in Portugal?		
Increasing	194	52.2%
Stable	161	43.3%
Decreasing	17	4.6%
What do you consider to be the class of drugs most often responsible for allergic reactions? *More than one possible option*		
Antibiotics	243	65.3%
Non-steroidal anti-inflammatory drugs	70	18.8%
Contrast media	37	9.9%
Cardiovascular medication	15	4%
Psychiatric and / or neurological medication	4	1.1%
Vaccines	2	0.5%
Vitamins	1	0.3%
Which consequences do you consider the most common after a drug allergy diagnosis? *More than one possible option*		
Patient insecurities in the future use of other medicines	309	83.1%
Difficulty in finding appropriate therapies	209	56.2%
Less therapeutic options	113	30.4%
Patient's quality of life	75	20.2%
Contraindication to some diagnostic tests	68	18.3%
Risk of death	106	28.5%
What percentage of risk of death exists because of drug allergy?		
0%	2	0.5%
≥ 1-5%	226	60.8%
> 5-10%	43	11.6%
> 10-20%	12	3.2%
> 20%	7	1.9%
I don't know	82	22%

In addition, this training showed no significant effect on the difficulty in distinguishing allergic reactions: 70.7% of non trained GPs found it difficult and 69.8% of those with training.

Discussion

Our sample allowed an acceptable accuracy level. However, we had expected a larger number of responses. The long and complicated bureaucratic process involved in the questionnaire distribution, out of our direct control, may have had a role in the low response rate. The identification of cutaneous signs as the most typical clinical manifestations of drug allergy by 65% of our responses corroborates several studies where the skin is the organ most frequently affected (15-17).

Moreover, according to our study, the drugs responsible for allergic reactions were mostly antibiotics and nonsteroidal anti-inflammatory drugs, in line with previous reports (12, 20, 21). However, the role of the latter is recognized only by 19% of GPs, being clearly underestimated by comparison with the available data (22). Allergy to antibiotics is perhaps overvalued not only in medical education, but also public opinion. Commonly, when faced with an allergic reaction, patients only remember their antibiotic intake. Similarly, to other studies (13, 23), more than a half of our study GPs perceived that drug allergy is increasing, which is understandable in the current setting of exponential medical drug consumption in Western society. In our study most of the doctors recognized drug allergy as a very important problem and revealed a good level of

Table II *- Drug allergy impact in GPs clinical practice (n = 372).*

	n	%
How do you evaluate the importance of drug allergy in your clinical practice? *Classify 1-4*		
1- Not Important	1	0.3%
2	27	7.3%
3	70	18.8%
4- Very important	274	73.7%
How many patients have evidence of drug allergy in your total patients list?		
< 10	97	26.1%
≥ 10-30	121	32.5%
≥ 30-50	52	14%
≥ 50-100	26	7%
≥ 100	13	3.5%
I don't know	63	16.9%
How often have you seen patients with drug allergy in the last year?		
Daily	3	0.8%
Weekly	43	11.6%
Monthly	146	39.2%
Twice a year	135	36.3%
Annually	36	9.7%
Never	9	2.4%
How many of these patients were investigated for drug allergy?		
None	102	27.4%
About 1/3	170	45.7%
About half	55	14.8%
About 2/3	21	5.6%
All	24	6.5%
In how many of these cases did this drug allergy have an impact on your therapeutic decision?		
None	11	3%
About 1/3	57	15.3%
About half	53	14.2%
About 2/3	30	8.1%
All	221	59.4%

awareness of the associated risk of fatal outcomes. A high impact in clinical practice is still reported, with 97.3% stating the need to use an alternative drug. The option not to medicate, in 6.7% of the patients, may represent a significant reduction in their quality of life, as it happens when painkillers are involved. In the case of antibiotics that option may carry a risk of a worse prognosis of infections.

In fact, adverse drug reactions affect 7% of general population, which represents an important cause of death. It is the sixth leading cause in United States and physicians often face the question whether a drug reaction is allergic and how it may affect patient care (24, 25).

Therefore, it would be expected that a large number of patients where there is a suspicion of a drug allergy would be referred for investigation. However, we observed that almost half of our responses estimated that only one third of these patients were referred for an allergy workup. Similar or even lower rates are reported by numerous other publications (26-29). In our opin-

Table III *- Drug allergy recognition and management (n = 372).*

	n	%
Which sign or symptom do you consider to be the most characteristic of drug allergy? *More than one possible option*		
Skin lesions	245	65.8%
Facial edema	55	14.8%
Itching	44	11.8%
Respiratory symptoms	28	7.5%
Change in blood pressure	3	0.8%
Malaise	6	1.6%
In cases where there was an implication in the therapeutic decision, you decided to: *More than one possible option*		
Do not medicate	25	6.7%
Lower the dose of medication	4	1.1%
Alternative drug	362	97.3%
Add antiallergic drug	29	7.8%
Other (refer to specialist)	3	0.8%
Do you usually refer patients without a confirmed diagnosis to Immunoallergology? *More than one possible option*		
All patients	82	22%
No patient	16	4.3%
Children	113	30.4%
When you don't have an alternative drug	152	40.9%
Only when the reaction is caused by certain drugs	26	7%
At patient's request	86	23.1%
Patients with an history of a severe reaction	255	68.5
Other (patients with an unclear history of drug allergy)	4	1.1%
Other (patients with multiple allergies)	1	0.3%
If you have selected "only when the reaction is caused by certain drugs", specify which drugs: *Open question*		
Antibiotics	9	2.4%
Betalactam antibiotics	3	0.8%
Angiotensin converting enzyme inhibitors	1	0.3%
Non-steroidal anti-inflammatory drugs	5	0.3%
Acetylsalicylic acid	1	0.3%
Allopurinol	1	0.3%
Antihistamines	1	0.3%
Allergy to multiple drugs	1	0.3%
Unanswered	4	1.1%
Do you find it easy in your clinical practice to distinguish an allergic reaction to a drug from a non-allergic reaction?		
Yes	111	29.8%
No	261	70.2%
During the past year, did you notify the National Pharmacovigilance System for adverse drug reactions (allergic or not) in your patients? *Note: it does not include notification in SClinic or other registration system used*		
Yes, I notified all reactions	25	6.7%
Yes, I notified the less usual and/or the most serious reactions	23	6.2%
Yes, I notified the reactions in that I considered very probable	44	11.8%
No, I never notified the National System	187	50.3%
Unanswered	93	25%

Table IV - *Referral to Immunoallergology consultations (n = 372).*

Currently in your clinical practice, how long does it take to have an Immunoallergology consultation requested by you to study drug allergy?										
	ARS Lisboa e Vale do Tejo		**ARS Centro**		**ARS Norte**		**ARS Alentejo**		**ARS Algarve**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
< 1 month	5	2.6%	3	3.6%	0	0%	1	4.2%	0	0%
1-3 months	70	35.7%	41	48.8%	19	31.6%	4	16.7%	4	50%
3-6 months	70	35.7%	29	34.5%	25	41.7%	4	16.7%	0	0%
> 6 months	51	26.0%	11	13.1%	16	26.7%	15	62.4%	4	50%
Are you satisfied with the Immunoallergology specialty collaboration in your area?										
	ARS Lisboa e Vale do Tejo		**ARS Centro**		**ARS Norte**		**ARS Alentejo**		**ARS Algarve**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Yes	113	57.6%	61	72.6%	38	63.3%	7	29.2%	5	62.5%
No	83	42.4%	23	27.4%	22	36.7%	17	70.8%	3	37.5%

Table V - *Training in drug allergy (n = 372).*

	n	**%**
Do you consider that the training in drug allergy is relevant to your clinical practice? *Classify 1-5*		
1- No interest	1	0.3%
2	0	0%
3	26	7%
4	113	30.4%
5- Very interesting	232	62.4%
Have you participated in any drug allergy training? *More than one possible option*		
University	98	26.3%
Postgraduate course	28	7.5%
Congress	107	28.8%
In our health center	28	7.5%
No	148	39.8%
Other (online curse, hospital fellowship as intern)	15	4%
Would you be interested in participating in training in this area?		
Yes	364	97.8%
No	8	2.2%
If so, what kind of training would you prefer?		
Online course	77	21.2%
Training actions in health centers	194	53.3%
Courses/workshops in national or regional congresses	37	10.2%
Discussion of cases with specialist regularly	54	14.8%
2nd and 4th options	1	0.3%
1st and 2nd options	1	0.3%

ion, GPs do not consider the immediate impact of using second line therapies (efficacy, adverse effects, antibiotic resistances), and more importantly do not perceive the need for long term clinical decisions.

The considerable delay in allergological evaluation, more striking in Alentejo, where the rate of Allergy specialists is the lowest in the country (0.59/100,000 inhabitants in Public Health Services according to the latest available data) (28), may be at least partially responsible for the insufficient investigations.

This delay is understandably related to physician satisfaction, according to our results, where centers reporting longer waiting times also reported higher rates of dissatisfaction. Clearly, the number of specialists is insufficient and should be increased.

In regards to GP education in drug allergy, about 40% of GPs had no specific training, the same rate was reported by Leru (13). This training is clearly insufficient, since more than two thirds of them mentioned difficulty in distinguishing allergic from non-allergic drug reactions. This same conclusion was reached by a survey undertaken by Yin Wang *et al.*, in Central China (25). Somewhat unexpected is that specific training did not improve the GPs' skills in drug allergy diagnosis, but a similar outcome was achieved by Sturmand and Temprano's survey at St. Louis University Medical Center, Missouri (USA) (24). This finding clearly calls into question the effectiveness of the training and strongly suggests that there should be an assessment of its quality by objectively measuring the results. Particularly, a more practical approach to learning should be emphasized. A positive attitude towards further training was expressed by almost all the GPs, in line with other published articles (13, 24).

Training sessions would be welcomed and locally delivered in health centers should be the chosen model. The need and interest for educational programs on drug allergy was also expressed in other European studies (12).

The use of an online questionnaire allowed us to gather information from all over the country in a practical, fast and inexpensive way, providing data that was easy to analyze and compare, while keeping the participant's anonymity. On the other hand, this methodology brought limitations to our study. Some of these limitations are those related to this type of study: the responses may not be completely truthful; there may be different interpretations of the questions, without the possibility of assuring a correct understanding; the emotional component of the answers cannot be perceived; the growing number of solicitations to respond to surveys may lead to a survey fatigue and a lower rate of response. Other limitations concerning specifically our study were the inexistence of a validated questionnaire for assessing educational needs in drug allergy and the bureaucratic difficulties faced in the distribution of questionnaires.

The results of our study suggest that GPs are generally aware of drug allergy as a problem, although sometimes fail to value some of the impacts of not investigating it. Additionally, we can conclude that the incidence of drug is perceived as an increasing phenomenon and the main culprits that were recognized were antibiotics and non-steroidal anti-inflammatory drugs. The study also reveals that the delayed response of Immunoallergology Departments in some regions accounts to some degree for GP dissatisfaction. Finally, this study highlights the need and the interest of family doctors in further drug allergy education, but quality criteria and practical issues should be reinforced in the training.

Fundings

None.

Contributions

BKC, LS, AMM: analysis conceptualization and design, data collection, analysis performance, writing - original draft. SMF, ET: analysis conceptualization and design, analysis performance, writing - original draft. TA, CM, FI: writing - review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

We would like to thank every ARS, ACES and Health Center coordinator that helped with the disclosure of the questionnaire.

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Appendix 1 *- Questionnaire.*

Dear colleagues,
Drug allergies are a pathology of supreme importance in the clinical practice of General Practitioners. As such, we kindly ask you to express your opinion in this area, taking into account the current clinical reality. Your answers are very important for the evaluation of this pathology in primary health care and will contribute to the identification of medical and educational training needs as well as the adequacy of the available information systems. The information obtained in this questionnaire is strictly confidential and only for scientific purposes.

Thank you for your cooperation!

☐ I agree that collected data in this anonymous questionnaire will be processed within the scope of this study.
Fields marked with (*) are required:

» **Professional experience** *
- Specialist with less than 5 years practice
- Specialist between 5 to 10 years practice
- Specialist with 10 to 20 years of practice
- Specialist with > 20 years of practice
- Trainees

» **Of which ARS do you belong to?** *
- ARS Norte
- ARS Centro
- ARS Lisboa e Vale do Tejo
- ARS Alentejo
- ARS Algarve

» **How do you describe the incidence of drug allergy in Portugal?** *
- Stable
- Increasing
- Decreasing

» **What do you consider to be the class of drugs most often responsible for allergic reactions? (More than one possible option)** *
- Antibiotics
- Non-steroidal anti-inflammatory drugs
- Cardiovascular medication
- Anti-inflammatory
- Vaccines
- Vitamins
- Psychiatric and/or neurological medication
- Contrast

» **Which consequences do you consider the most common after a diagnosis of drug allergy? (More than one possible option)** *
- Patient insecurities in the future use of other medicines
- Risk of death
- Less therapeutic options
- Difficulty in finding appropriate therapies
- Contraindication to some diagnostic tests
- Other: _____

» **What percentage of risk of death exists because of drug allergy?** *
- 0%
- > 1-5%
- > 5-10%
- > 10-20%
- > 20%
- I don't know

» **How do you evaluate the importance of drug allergy in your clinical practice?** *
- 1- No important 2 3 4 5-Very important

» **How many patients have evidence of drug allergy in your total patients list?** *
- < 10
- ≥ 10-30
- ≥ 30-50
- ≥ 50-100
- ≥ 100
- I don't know

» **How often have you seen patients with drug allergy in the last year?** *
- Daily
- Weekly
- Monthly
- Twice a year
- Annually
- Never

» **How many of these patients were investigated for drug allergy?** *
- None
- About 1/3
- About half
- About 2/3
- All

» **In how many of these cases did this drug allergy have an impact on your therapeutic decision?** *
- None
- About 1/3
- About half
- About 2/3
- All

» **Which sign or symptom do you consider to be the most characteristic of drug allergy? (More than one possible option)** *
- Skin lesions
- Facial edema
- Itching
- Respiratory symptoms
- Change in blood pressure
- Indisposition
- Other: _____

» **In cases where there was an implication in the therapeutic decision, you decided to: (More than one possible option)** *
- Do not medicate
- Lower the dose of medication
- Alternative drug
- Add antiallergic drug
- Other: _____

» **Do you usually refer patients without a confirmed diagnosis to Immunoallergology? (More than one possible option)** *
- All patients
- No patient
- Children
- When you don't have an alternative drug
- Only when the reaction is caused by certain drugs
- At patient´s request
- Other: _____

» **If you have selected the option "only when the reaction is caused by certain drugs", specify which: _____**

» **Do you find it easy in your clinical practice to distinguish an allergic reaction to a drug from a non-allergic reaction?** *
- Yes
- No

» **During the past year, did you notify the National Pharmacovigilance System for adverse drug reactions (allergic or not) in your patients? Note: Does not include notification in SClinic or other registration system used.** *
- Yes, I notified all reactions
- Yes, I notified less usual and/or the most serious reactions
- Yes, I notified the reactions in that I considered very probable
- No, I never notified the National System

» **Currently in your clinical practice, how long does it take to have an Immunoallergology consultation requested by you to study drug allergy?** *
- < 1 month
- 1-3 months
- 3-6 months
- > 6 months

» **Are you satisfied with the Immunoallergology specialty collaboration in your area?** *
- Yes
- No

» **Do you consider that the training in drug allergy is relevant to your clinical practice?** *
- 1- No important 2 3 4 5-Very important

» **Have you participated in any drug allergy training? (More than one possible option)** *
- University
- Postgraduate course
- Congress
- In our health center
- No
- Other: _____

» **Would you be interested in participating in training in this area?** *
- Yes
- No

» **If so, what kind of training would you prefer?** *
- Online course
- Training actions in health centers
- Courses/ workshops in national or regional congresses
- Discussion of cases with specialist regularly
- 2nd and 4th options
- 1st and 2nd options

Eur Ann Allergy Clin Immunol Vol 55, N.2, 68-77, 2023

Martin Berge[1], Lena Bertilsson[2], Olof Hultgren[3], Svante Hugosson[4], Amanj Saber[4]

Qualitative and quantitative comparison of allergen component-specific to birch and grass analyzed by ImmunoCAP assay and Euroline immunoblot test

[1]Department of Otolaryngology, Örebro University Hospital, Örebro, Sweden
[2]Department of Laboratory Medicine, Örebro University Hospital, Örebro, Sweden
[3]Department Clinical Immunology and Transfusion Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden
[4]Department of Otolaryngology, Örebro University Hospital, School of Medial Sciences, Faculty of Medicine and Health, Örebro University, Örebro, Sweden

Key words

Timothy; birch; pollen; allergen-specific IgE; ImmunoCAP; Euroline.

Corresponding author
Amanj Saber
Department of Otolaryngology
Örebro University Hospital
School of Medial Sciences
Faculty of Medicine and Health
Örebro University
SE 70182 Örebro, Sweden
ORCID: 0000-0002-4141-4256
E-mail: amanj.saber@regionorebrolan.se

Doi
10.23822/EurAnnACI.1764-1489.241

Summary

Background. *In the diagnostic work up of allergy, determining allergen component-specific immunoglobulin E (IgE) is important for diagnosis, prognosis and choice of treatment. The purpose of this study was to evaluate the performance of the immunoblotting assay (Euroline) in detection of IgE antibodies against timothy grass and birch pollen allergen components compared to fluorescent enzyme assay (ImmunoCAP, Phadia 250).* ***Methods.*** *A total of 128 serum samples from patients allergic to timothy grass and birch pollen were analyzed. The levels of IgE antibodies to timothy grass and birch pollen were measured using Euroline DPA-Dx pollen 1 and ImmunoCAP assay. The two methods were then compared on binary (positive vs negative), semi-quantitative (IgE classes) and quantitative (concentration) levels. The two methods were also compared to results from skin prick testing.* ***Results.*** *The Euroline method showed a positive percentage agreement of 93% and negative percentage agreement of 94% with an overall accuracy of 94% when compared to ImmunoCAP. Kappa analysis showed moderate strength of agreement between the methods in determining IgE classes for 7/11 components tested. All components showed a positive correlation when analysed using Spearman's rank correlation.* ***Conclusions.*** *Overall, we found that there is good correlation between the Euroline and ImmunoCAP methods in measuring IgE sensitization.*

Impact statement

Use of the Euroline method may be recommended as an alternative routine clinical allergy diagnostic work up to determine sensitization profiles to timothy grass and birch pollen.

Introduction

It is challenging to diagnose allergic diseases especially when there are contradictory outcomes between clinical and laboratory findings. Thus, correct diagnosis requires good agreement between the clinical features and serological tests. Accurately detected specific Immunoglobulin E (IgE) sensitization and specific IgE profiling is not only essential to diagnose allergic patients, but it also has a key impact on optimal decision making for successful allergen immunotherapy strategies tailored for the individual patient (1). The use of allergen components is of great diagnostic importance for identifying the major sensitizing component, especially when the results of allergen-specific IgE mismatch with the subjective allergic symptoms of the patient. As a result, the risk of serological cross-reaction and/or over-interpretation of the results is reduced.

Various methods exist for testing allergen-specific IgE antibodies and their results can vary greatly, thereby affecting both the diagnosis and treatment of allergic diseases (2). It is not easy to compare the results of different test systems with each other as there are differences in the development of the methods. An ideal assay method would be simple and easily carried out, time- and cost effective, and most importantly, of the highest performance. The singleplex immunoassay ImmunoCAP (Thermo Fischer) specific IgE measuring assay system is used worldwide as a diagnostic test for allergy. It is the most widely evaluated method and its sensitivity, specificity, and positive predictive values have been shown to be over 90%, while other methods are less focused (1). However, the multiparameter assays for specific IgE assay (Euroline), are used with increasing frequency (3, 4). This test system offers the advantage that allergen-specific IgE antibodies, against multiple pollen allergens, can be determined semi-quantitatively in a single serum incubation.

The aim of this study is to describe the effectiveness of Euroline compared to ImmunoCAP in identifying levels of allergen-specific IgE antibodies to timothy grass and birch pollen, and to evaluate Euroline as a potential alternative method for routine clinical testing of allergen-specific IgE. The two methods will be compared in relation to the subjects' allergic profiles, as well as how the subjects have responded to allergen immunotherapy (AIT).

Materials and methods

Study design and population

This study is a retrospective study based on a cohort of 128 adult patients with medical history of allergic rhinitis, positive skin prick tested (SPT) and/or allergen-specific IgE test for timothy grass and/or birch pollen allergy who were set to undergo AIT targeting grass and/or birch allergy. This cohort has been used previously by our group (4). This study has received approval by the Swedish Ethical Review Authority.

Skin prick test

SPTs were carried out using a panel of commercially available extracts (Soluprick SQ®, ALK – Abelló; Hørsholm, Denmark).

Detection of the allergen components

Serum from the 128 subjects was analyzed using two different methodologies (ImmunoCAP and Euroline). As the testing occurred prior to designing this study, there is some mismatch between the components tested with each method. Only subjects where there was enough serum sample to perform analysis with both methods were included in the analysis of each specific allergen component. A total of 1,364 paired tests were performed on the 128 serum specimens.

Method 1 (ImmunoCAP, Phadia 250)

Serum samples stored at -20 °C were analyzed for allergen-specific IgE antibodies with ImmunoCAP Fluoro Enzyme Immuno Assay (FEIA) (Thermo Fisher Scientific/Phadia AB, Uppsala, Sweden) according to the manufacturer's recommendations. The samples were analyzed for specific IgE against *Phleum pratense*, timothy (g6) and its allergenic components rPhl p 1, rPhl p 4, rPhl p 5b, rPhl p 6, rPhl p 7+rPhl p 12; against *Betula verrucosa*, birch (t3) and its allergenic components rBet v 1, rBet v 6, rBet v 2+rBet v 4 and MUXF3 CCD, Bromelin. These components for birch and timothy pollen allergy were chosen according to Thermo Fisher's recommendation. The result for each allergen is stated in kU/L, the limit of detection is 0.1 kU/L and is divided into the following classes: 0 (< 0.35 kU/L), 1 (0.35 kU/L to < 0.7 kU/L), 2 (0.7 kU/L to < 3.5 kU/L), 3 (3.5 kU/L to < 17.5 kU/L), 4 (17.5 kU/L to < 50 kU/L), 5 (50 kU/L to < 100 kU/L) and 6 (≥ 100 kU/L). Controls for timothy and birch with known concentrations have been run at each analysis to ensure that the method works as intended.

Method 2 (EUROBlotOne, Euroline DPA-Dx pollen 1)

Serum-specific IgE antibodies were measured with EUROBlotOne, EUROLINE DPA-Dx pollen 1 (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) according to the manufacturer's instructions, and the results expressed in kU/L with a lower limit of detection of 0.35 kU/L and an upper limit of 100 kU/L. The results were divided into classes in the same way as method 1. The test kit contained strips marked with parallel lines with 11 different allergens and a control line (indicator band). Serum samples were analyzed for specific IgE against *Betula verrucosa*, birch (t3) and the birch components rBet v 1, rBet v 2, rBet v 4, rBet v 6; *Phleum pratense*, timothy (g6) and the timothy components rPhl p 1, rPhl p 5, rPhl p 7, rPhl p 12 and the cross-reactive carbohydrate determinants (CCDs). A known control sample, positive for birch (t3) and timothy (g6) was run with each analysis to ensure that the method worked as intended.

Statistics

Microsoft® Excel® was used to store the data and to create the tables presented in this study. All interval or ordinal level variables are presented as median (min-max). Categorical level variables are presented as frequencies (percentage).

All statistical analysis was conducted in R, a software environment for statistical computing (5). To compare the two methods' agreements in determining the semi-quantitative classes of specific IgE concentration, Cohen's kappa values were calculated (6). To compare the performance between the two methods of measuring the concentration of specific IgE, a Spearman correlation analysis was performed (7). To visualize the quantitative agreement between the two methods, both conventional scatter plots, as well as Bland-Altman plots were constructed (8). As a crude measure of clinical significance, sIgE levels were compared to AIT outcome using Fisher's exact test, as previously done by the authors for the Euroline test method (4).

As the lower and upper limits of detection are different for the two methods, IgE concentration value < 0.35 kU/L was converted to 0.34 kU/L and value ≥ 100 kU/L was converted to 100 kU/L prior to statistical testing.

Results

Out of the 128 study subjects, 77 (60%) were female. The median age of the patients was 33 years, ranging from 17 to 70 years. A total of 61 (48%) subjects reported suffering from asthma, while 34 (27%) patients had an asthma diagnosis according to the medical records. Positive skin prick tests (SPT) for timothy grass were seen in 114 patients, and for birch in 105 patients. In three patients, information about SPT results could not be found in the medical records. One patient had tested negative with SPT for both timothy grass and birch. The vast majority of the patients were polysensitized (n = 117) with a positive SPT for two or more allergens (grass and birch, or grass or birch plus some other allergen). Baseline characteristics of the study subjects are shown in **table I**. The overall accuracy of Euroline compared to ImmunoCAP was 94%, with a positive percentage agreement (PPA) of 93% and negative percentage agreement (NPA) of 95%. The lowest PPA was seen when comparing Euroline Phl p 7 to ImmunoCAP Phl p 7+Phl p 12 (20%) and Euroline Bet v 4 to ImmunoCAP Bet v 4+Bet v 2 (17%). The performance of Euroline assays compared to ImmunoCAP on a nominal level (positive *vs* negative), can be seen in **table IIa**. Similar high performance was observed when positive IgE to g6 and t3 was compared to grass and birch sensitization according to SPT (**table III**).

For cross-reactive carbohydrate determinants (CCDs), the number of positive subjects was generally low for both test methods. 7 subjects were positive for CCDs according to both Euroline and ImmunoCap, while 10 was only positive according Euroline and 1 only according to ImmunoCAP (positive predictive value, PPV 41%). However, the overall accuracy of Euroline in determining positivity for CCDs was high (91%). For the 11 subjects with discordant results on the CCD analysis, no obvious differences were seen for the other components (**table IIb**). There were 9 subjects who were positive for Bet v1 according to Euroline while negative according ImmunoCap. Out of these 9 subjects, 5 had shown positive SPT to birch.

To assess the inter-rater agreement between Euroline and ImmunoCAP assays in determining the semi-quantitative classes of IgE concentration, weighted Cohen's kappa coefficients (κ_w value) were calculated (**figure 1**). The lowest κ_w value was observed for the comparison of Euroline v4 to ImmunoCAP v 4+v 2 (0.27; 0.20-0.34) and the highest for Euroline Bet v 6 to ImmunoCAP Bet v 6 (0.74; 0.66-0.83).

Spearman rho coefficients (r_s) between the two methods were calculated for each component (**figure 2**). This analysis showed positive r_s values with P-values < 0.05 for all components tested. The lowest r_s values were found when comparing Euroline Phl p 7 to ImmunoCAP Phl p 12+Phl p 7 ($r_s = 0.44$, $p < 0.001$) and Euroline Bet v 4 to ImmunoCAP Bet v 4+Bet v 2 ($r_s = 0.39$, $p < 0.001$). The highest r_s value was found when comparing Euroline Phl p5 to ImmunoCAP Phl p5 and Euroline Bet v1 to ImmunoCAP Bet v1 ($r_s = 0.96$, $p < 0.001$). As shown in **figure 2**, Euroline showed a positive bias compared to ImmunoCap for most of the tested components.

There were no statistically significant differences between AIT outcomes and IgE-levels as measured by ImmunoCAP (**table IV**).

Discussion

Proper diagnosis of clinical allergy and effective treatment are critical for patients with allergy (9). This requires correct diagnosis of an IgE-mediated disease and a clear connection between the identified allergen and the patient's symptoms.

Table I *- Baseline characteristics of the study population (n = 128).*

		n (%)
Age	< 30	56 (44)
Sex	Female	77 (60)
Asthma	Diagnosed	34 (27)
	Self-reported	61 (47)
SPT	Only grass	19 (15)
	Only birch	10 (8)
	Grass and birch	95 (74)
	Polysensitized[a]	117 (91)
Symptom severity[b]	Moderate	7 (5)
	Severe	121 (95)

SPT: skin prick test; [a]positive SPT to more than one allergen; [b]measured by numeric rating scale where 2-5 is considered moderate and > 5 as severe.

Table II *- **(a)** Performance of Euroline method in measuring sensitization to different allergen specific IgE molecules, compared to ImmunoCAP method; **(b)** Only including subjects with discordant results for CCD.*

(a)										
Component	**n**	**E^+/IC^+**	**E^+/IC^-**	**E^-/IC^+**	**E^-/IC^-**	**PPA**	**NPA**	**PPV**	**NPV**	**Accuracy**
p12 *vs* p12+p7	125	12	3	3	107	80%	97%	80%	97%	95%
p7 *vs* p12+p7	125	3	0	12	110	20%	100%	100%	90%	90%
p5	125	83	3	0	39	100%	93%	97%	100%	98%
p1	128	103	4	0	21	100%	84%	96%	100%	97%
v6	123	10	0	4	109	71%	100%	100%	96%	97%
v4 *vs* v4+v2	125	2	0	10	113	17%	100%	100%	92%	92%
v2 *vs* v4+v2	125	10	8	2	105	83%	93%	56%	98%	92%
v1	125	97	9	0	19	100%	68%	92%	100%	93%
g6	121	101	4	3	13	97%	76%	96%	81%	94%
t3	121	98	1	4	18	96%	95%	99%	82%	96%
CCD	123	7	10	1	105	88%	91%	41%	99%	91%
Total	**1366**	**526**	**42**	**39**	**759**	**93%**	**95%**	**93%**	**95%**	**94%**
(b)										
Component	**n**	**E^+/IC^+**	**E^+/IC^-**	**E^-/IC^+**	**E^-/IC^-**					
p12 *vs* p12+p7	11	1	0	1	9					
p7 *vs* p12+p7	11	1	0	1	9					
p5	11	11	0	0	0					
p1	11	11	0	0	0					
v6	11	1	0	0	10					
v4 *vs* v4+v2	11	1	0	1	9					
v2 *vs* v4+v2	11	2	0	0	9					
v1	11	10	0	0	1					
g6	10	10	0	0	0					
t3	10	9	0	0	1					
CCD	11	0	10	1	0					
Total	**119**	**57**	**10**	**4**	**48**					

E: Euroline; IC: ImmunoCAP; PPA: positive percentage agreement; NPA: negative percentage agreement; PPV: positive predictive value; NPV: negative predictive value.

Figure 1 *- Inter-rater agreement between Euroline and ImmunoCAP methods for determining IgE classes (0-6) to different allergen specific IgE.*

Phl p12 vs Phl p12+p7

κw (95% CI) = 0.55 (0.46-0.64)

Euroline Phl p12 \ ImmunoCap Phl p12+p7	0	1	2	3	4	5	6
6	0	0	0	0	0	0	0
5	0	0	0	2	0	0	0
4	0	0	1	0	0	0	0
3	1	1	2	0	0	0	0
2	1	5	1	0	0	0	0
1	1	0	0	0	0	0	0
0	**107**	1	0	2	0	0	0

n = 125

Phl p7 vs Phl p12+p7

κw (95% CI) = 0.32 (0.25-0.4)

Euroline Phl p7 \ ImmunoCap Phl p12+p7	0	1	2	3	4	5	6
6	0	0	0	2	0	0	0
5	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
2	0	1	0	0	0	0	0
1	0	0	0	0	0	0	0
0	**110**	6	4	2	0	0	0

n = 125

Phl p5

κw (95% CI) = 0.58 (0.34-0.81)

Euroline \ ImmunoCap	0	1	2	3	4	5	6
6	0	0	0	1	8	7	4
5	0	0	5	**32**	11	1	0
4	0	0	6	3	0	0	0
3	0	1	3	0	0	0	0
2	1	1	0	0	0	0	0
1	2	0	0	0	0	0	0
0	**39**	0	0	0	0	0	0

n = 125

Phl p1

κw (95% CI) = 0.34 (0.12-0.56)

Euroline \ ImmunoCap	0	1	2	3	4	5	6
6	0	0	0	**26**	**23**	6	3
5	0	0	15	18	2	1	1
4	0	1	5	0	0	0	0
3	1	1	0	0	0	0	0
2	2	0	0	0	0	0	0
1	1	0	1	0	0	0	0
0	**21**	0	0	0	0	0	0

n = 128

Bet v6

κw (95% CI) = 0.74 (0.66-0.83)

Euroline \ ImmunoCap	0	1	2	3	4	5	6
6	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
4	0	0	0	1	0	0	0
3	0	0	0	2	0	0	0
2	0	0	2	3	0	0	0
1	0	0	1	1	0	0	0
0	**109**	2	2	0	0	0	0

n = 123

Bet v4 vs Bet v4+v2

κw (95% CI) = 0.27 (0.2-0.34)

Euroline Bet v4 \ ImmunoCap Bet v4+v2	0	1	2	3	4	5	6
6	0	0	0	0	0	0	0
5	0	0	0	1	0	0	0
4	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0
0	**113**	4	4	2	0	0	0

n = 125

Bet v2 vs Bet v4+v2

κw (95% CI) = 0.45 (0.36-0.54)

Euroline Bet v2 \ ImmunoCap Bet v4+v2	0	1	2	3	4	5	6
6	0	0	0	0	0	0	0
5	0	0	1	2	0	0	0
4	0	1	2	0	0	0	0
3	2	2	0	0	0	0	0
2	2	1	0	1	0	0	0
1	4	0	0	0	0	0	0
0	**105**	1	1	0	0	0	0

n = 125

Bet v1

κw (95% CI) = 0.38 (0.17-0.59)

Euroline \ ImmunoCap	0	1	2	3	4	5	6
6	0	0	0	3	7	8	2
5	0	0	6	**41**	14	2	0
4	0	2	8	0	0	0	0
3	3	4	0	0	0	0	0
2	3	0	0	0	0	0	0
1	3	0	0	0	0	0	0
0	**19**	0	0	0	0	0	0

n = 125

g6

κw (95% CI) = 0.47 (0.25-0.69)

Euroline \ ImmunoCap	0	1	2	3	4	5	6
6	0	0	0	0	13	9	8
5	0	0	1	**29**	**23**	1	1
4	0	1	3	4	1	0	0
3	0	1	3	1	0	0	0
2	1	1	0	0	0	0	0
1	3	1	0	0	0	0	0
0	**13**	1	2	0	0	0	0

n = 121

t3

κw (95% CI) = 0.52 (0.3-0.74)

Euroline \ ImmunoCap	0	1	2	3	4	5	6
6	0	0	0	0	1	1	3
5	0	0	2	**27**	**28**	**13**	3
4	0	0	6	1	0	0	0
3	0	4	4	0	0	0	0
2	1	3	1	0	0	0	0
1	0	0	0	1	0	0	0
0	**18**	2	2	0	0	0	0

n = 121

CCD

κw (95% CI) = 0.49 (0.41-0.58)

Euroline \ ImmunoCap	0	1	2	3	4	5	6
6	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
4	0	0	4	0	0	0	0
3	1	0	2	0	1	0	0
2	3	0	0	0	0	0	0
1	6	0	0	0	0	0	0
0	**105**	1	0	0	0	0	0

n = 123

Interpretation of Cohen's K coefficients (Kw):

≤ 0.20 no or slight agreement
0.21-0.40 fair agreement
0.41-0.60 moderate agreement
0.61-0.80 substantial agreement
≥ 0.81 almost perfect agreement

Numbers in bold represents IgE classes and numbers in central grid represent frequencies; κ_w: weighted Cohen's kappa coefficient; CI: confidence interval.

Table III *- Performance of Euroline and ImmunoCAP methods in measuring sensitization to different allergens, compared to SPT.*

IgE assay	n	IgE^+/SPT^+	IgE^+/SPT^-	IgE^-/SPT^+	IgE^-/SPT^-	Sensitivity	Specificity	PPV	NPV	Accuracy
Euroline Grass	128	108	3	6	11	95%	79%	97%	65%	93%
Euroline[a] Grass	121	102	3	6	10	94%	77%	97%	63%	93%
ImmunoCAP Grass	121	102	2	6	11	94%	85%	98%	65%	93%
Euroline Birch	128	98	7	7	16	93%	70%	93%	70%	89%
Euroline[a] Birch	121	93	6	7	15	93%	71%	94%	68%	89%
ImmunoCAP Birch	121	95	7	5	14	95%	67%	93%	74%	90%

IgE: Immunoglobulin E; SPT: skin prick test; PPV: positive predictive value; NPV: negative predictive value; [a]only including patients with a valid ImmunoCAP test result.

The singleplex assay ImmunoCAP system has high analytical sensitivity (lower limit of quantitation) and greater sensitivity at low specific IgE levels. It needs only 40 µL serum or plasma per individual test (1). However, it can be criticized for being expensive, requiring individualized testing and having a lengthy testing time (10). As an alternative, a single test for multiple allergens using customized allergen profiles (Euroline) has been introduced in clinical practice as a reliable and cost-efficient specific IgE test with acceptable correlation with ImmunoCAP. It is simpler and faster than the ImmunoCAP system, and requires a small serum volume (100-200 µL) to provide results for multiple allergen components and to get a screening overview of the patient's sensitization (3, 11, 12). The method is gaining increasing clinical awareness (3, 4). However, when looking at scientific publications in the Medline database published before 2020, ImmunoCAP heavily outweighs Euroline with 600 publications compared to 7 articles about Euroline (2).

Because of the underlying different methodological backgrounds, it is not surprising that differences appear between different immunoassays in terms of sensitivity and specificity or in terms of IgE concentration. These may be due to differences in method sensitivity, the use of native or recombinant allergens and the representation of the sensitizing molecule in the testing procedure (1, 13). This may cause some confusion when it comes to the interpretation of the test results. Hence, we evaluated the allergen-specific IgE antibody-detection performance of the Euroline immunoblot test in comparison to ImmunoCAP system.

Our study found that on a binary level (*i.e.*, positive *vs* negative) the Euroline test method has good concordance with the ImmunoCAP method. The PPA of Euroline compared to ImmunoCAP was > 80% for 8/11 tested molecules, and the NPA > 80% in 9/11 molecules. The cumulative PPA and NPA for all molecules were 93% and 95%, respectively. The cumulative accuracy in comparison to ImmunoCAP was 94%. These findings indicate that the Euroline method is reliable in testing specific IgE sensitization and is in accordance with previous studies (1, 3).

To make the comparison more impartial and interesting, we tested how these two methods performed when compared to results from SPT. When comparing SPT positivity for grass to IgE positivity for g6 and SPT positivity for birch to IgE positivity for t3, both the Euroline and ImmunoCAP methods showed similar accuracy (**table III**). This outcome outlines that the Euroline method is a reliable alternative method to the gold standard method ImmunoCAP in testing for IgE sensitization.

When comparing the IgE classes as determined by the two methods, the κ_w value was > 0.40 for 7/11 subjects. The highest κ_w value was found for v6 (0.74). According to a traditional interpretation of kappa coefficients a value > 40 would be considered a moderate strength of agreement, while 0.74 would be considered a substantial strength of agreement. However, some researchers deem this interpretation to attribute too much strength to low coefficients. According to McHugh (14) a kappa coefficient > 40 should instead be considered a weak level of agreement and > 60 as a moderate level of agreement.

When comparing IgE concentrations as determined by the two methods, all tested allergen components showed a positive r_s value with a P-value < 0.05, indicating that there was indeed a positive correlation. Further, 6/11 tested allergen components showed r_s values > 0.8, which can be described as a very strong relationship (15). Only two of the tested components showed r_s values between 0.3 and 0.5, which can be considered a fair strength of relationship. Furthermore, these two r_s values were found when comparing a single component tested with Euroline (Phl p 7 and Bet v 4) with multiple components tested with ImmunoCAP (Phl p 12+Phl p 7 and Bet v 4+Bet v 2). These findings can potentially be related to the fact that two different sets of components were compared, as a single component in Euroline was compared to a combination of two components in ImmunoCAP. These outcomes could have been quite different if

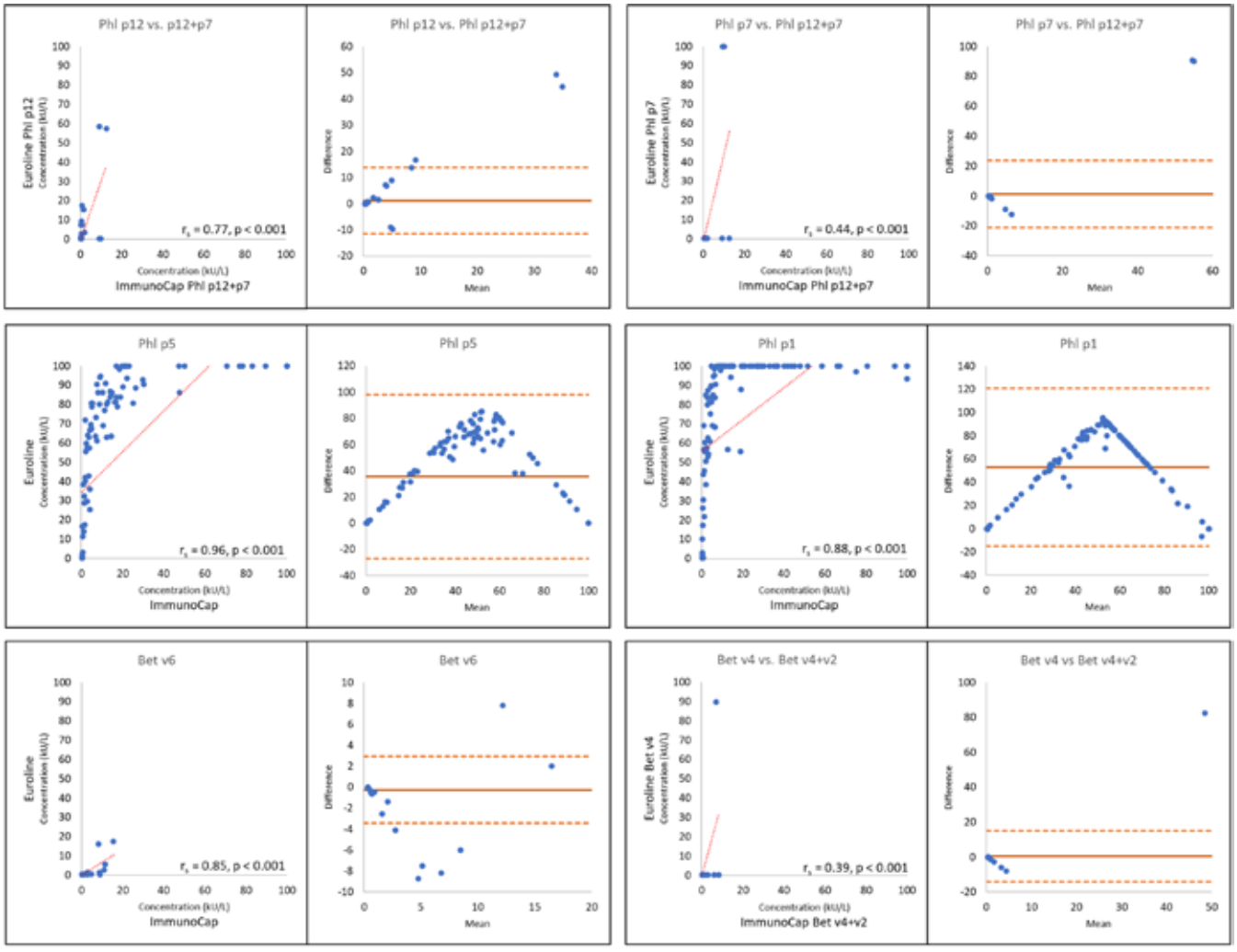
ImmunoCAP's single component analysis for Phl p 12, Phl p 7, Bet v 4, and Bet v 2 components had been used instead.

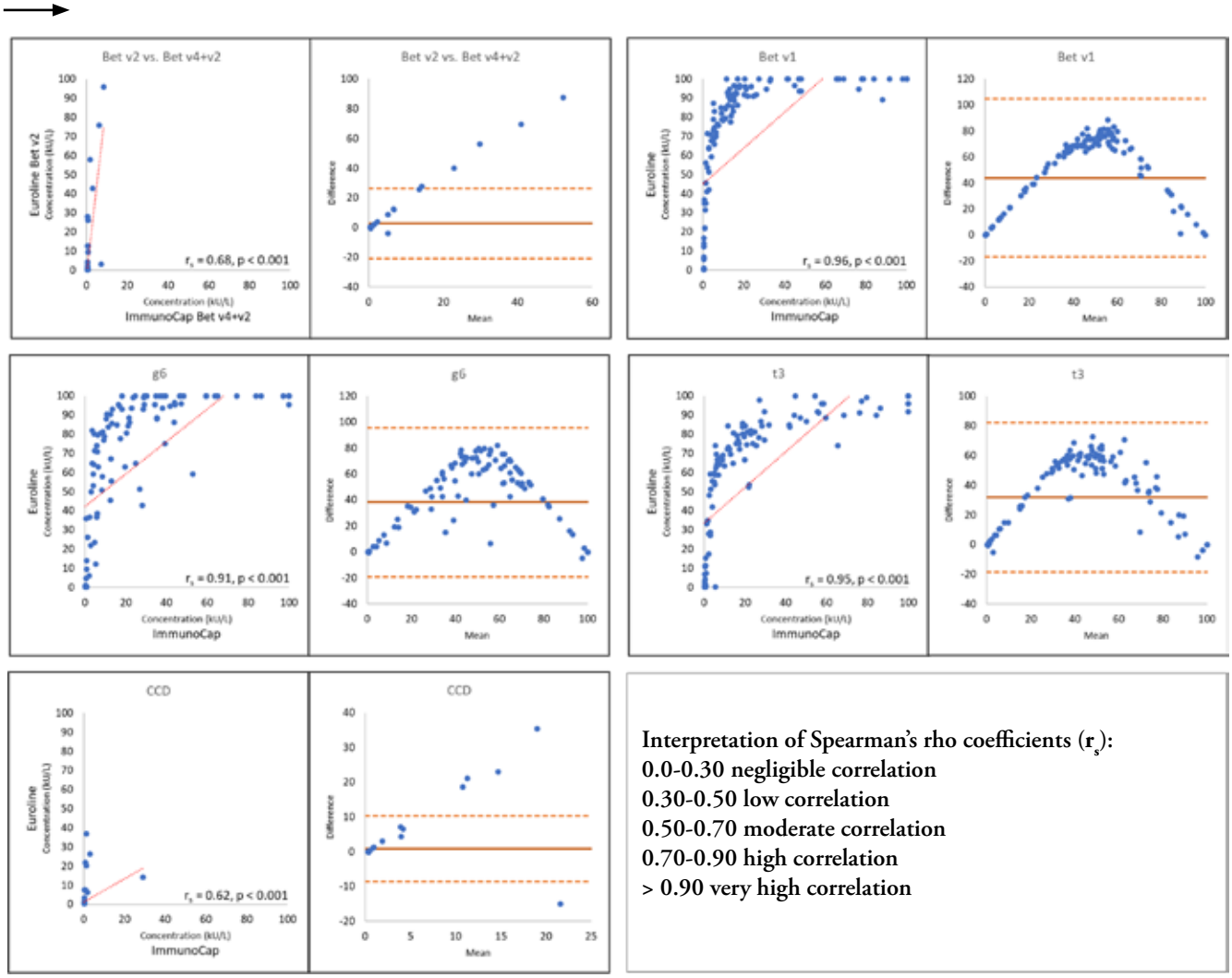
No clear association was found between AIT outcome and pre-treatment IgE levels as measured by ImmunoCAP (**table IV**). This is similar to the results previously published by the authors concerning association between AIT outcome and IgE levels as measured by Euroline (4).

An advantage with the Euroline immunoblot test is that the strip includes the cross-reactive carbohydrate determinants (CCD) marker; with ImmunoCAP, CCD-specific IgE must be tested separately. CCD is present in many allergens with reactivity in 7.5-35% of the patients. It has little clinical relevance, but can be a problem in diagnosis, as the presence of IgE towards CCDs may cause a false-positive reaction due to interference or cross-reactivity in allergen-specific IgE assays (2). Therefore, the CCD marker may provide useful information, especially with positive-specific IgE results that disagree with the clinical picture and can aid in interpreting overall test results showing concurrent positivity for multiple allergens (12, 16).

Although most samples (91%) show qualitatively the same anti-CCD results according to both Euroline and ImmunoCap methods, there were eleven samples (9%) with discordant results on a negative to positive (> 0.35 kU/L)-scale (**table IIa**). In one way it is preferable not to find too many positive CCD-reactions since a true CCD-result may cast some shadow on the clinical importance on the results for naturally derived allergens

Figure 2 *- Correlation between concentration of allergen-specific IgE, as measured by either Euroline or ImmunoCAP method.*





Presented with scatter plots visualizing the concentration according to the different methods on the left, and Bland Altman plots to the right; r_s: Spearman's rho coefficient.

in the same sample, while that is not a problem for recombinant allergen components. On the other hand, it is preferable to receive a CCD-reaction, if it is a true one, if the presence of anti-CCD antibodies may be a reason for false positive reactions in analyses for single allergens in the same sample. False positive reactions to single allergens may result in a false clinical interpretation and unnecessary avoidance of harmless allergens.

There are different approaches how to handle a positive CCD result in clinical routine situations. One way is to make further analyses with recombinant components, another way is an absorption of anti-CCD antibodies before analysis of IgE against the single allergens. In some cases, a "second opinion" by another method may be valuable. However, if it is a true anti-CCD reaction and both sources of naturally derived allergens contain anti-CCD antibodies this way may not be a valuable option. In most clinical situations the results from analysis of IgE to allergens are considered together with the clinical context, and if valuable and possible a common choice is analyses with recombinant allergen components.

For the 11 subjects with discordant results to CCD, there were no obvious increase in discordant results for the other analysed components (**table IIb**). This was to be expected as all single components analysed in this study were recombinant allergen components.

Visual inspection of **figures 1** and **2** shows that on both a semi-quantitative and quantitative level (*i.e.*, measuring IgE classes and IgE concentration, respectively) the Euroline method tended to show higher values compared to the ImmunoCAP method. This is shown clearly in the Bland-Altman plots presented in **figure 2**, where Euroline shows a positive bias compared to ImmunoCap for most of the tested components. This

Table IV *- Comparison of specific immunoglobulin E (IgE) levels as measured by ImmunoCAP between the study subjects, stratified based on response to allergen immunotherapy.*

Component	n	Concentration (kU/L)	Non-improved n (%)	Improved n (%)	P-value
Phl p12 + Phl p7	125	< 0.35	33 (26%)	77 (62%)	0.792
		0.35-3.4	2 (2%)	9 (7%)	
		3.5-49.9	1 (1%)	3 (2%)	
		> 50	0 (0%)	0 (0%)	
Phl p5	125	< 0.35	16 (13%)	26 (21%)	0.555
		0.35-3.4	4 (3%)	12 (10%)	
		3.5-49.9	14 (11%)	41 (33%)	
		> 50	4 (3%)	8 (6%)	
Phl p1	128	< 0.35	6 (5%)	19 (15%)	0.804
		0.35-3.4	8 (6%)	15 (12%)	
		3.5-49.9	20 (16%)	49 (38%)	
		> 50	4 (3%)	7 (5%)	
g6	121	< 0.35	4 (3%)	13 (11%)	0.856
		0.35-3.4	5 (4%)	9 (7%)	
		3.5-49.9	19 (16%)	52 (43%)	
		> 50	6 (5%)	13 (11%)	
Bet v6	123	< 0.35	34 (28%)	75 (61%)	0.578
		0.35-3.4	1 (1%)	6 (5%)	
		3.5-49.9	1 (1%)	6 (5%)	
		> 50	0 (0%)	0 (0%)	
Bet v4 + Bet v2	125	< 0.35	34 (27%)	79 (63%)	1.000
		0.35-3.4	2 (2%)	7 (6%)	
		3.5-49.9	1 (1%)	2 (2%)	
		> 50	0 (0%)	0 (0%)	
Bet v1	125	< 0.35	11 (9%)	17 (14%)	0.154
		0.35-3.4	5 (4%)	15 (12%)	
		3.5-49.9	15 (12%)	50 (40%)	
		> 50	6 (5%)	6 (5%)	
t3	121	< 0.35	8 (7%)	11 (9%)	0.333
		0.35-3.4	6 (5%)	18 (15%)	
		3.5-49.9	13 (11%)	45 (37%)	
		> 50	7 (6%)	13 (11%)	
CCD	123	< 0.35	34 (28%)	81 (66%)	1.000
		0.35-3.4	2 (2%)	5 (4%)	
		3.5-49.9	0 (0%)	1 (1%)	
		> 50	0 (0%)	0 (0%)	

IgE levels are presented as frequency (%), with P-values from Fisher's exact test.

finding might be due to manufacturing and calibration differences between the two methods (3). A difficulty in comparing these two methods is that the two methods have different limits of detection. Euroline only measures IgE concentrations between 0.35 kU/L and 100 kU/L, while ImmunoCAP measures IgE concentrations > 0.1 kU/L. In clinical practice, concentration values below 0.35 kU/L are usually considered as negative, class 0; however, this is not an absolute limit but rather expresses a probability of allergy. Thus, it could be considered of importance to be able to measure concentrations less than 0.35 kU/L. Overall, caution should be exercised when comparing results measured with different methods. Thus, despite the relatively good correlation between these two methods that has been shown in this study, there may be other discrepancies between these test systems which could not be ruled out.

According to the findings of the present study, we conclude that the Euroline method performs well and is concordant with ImmunoCAP in determining sensitization to birch and grass pollen allergen molecules. Furthermore, Euroline shows acceptable correlation to ImmunoCAP in determining specific IgE concentration for birch and grass pollen molecules. Use of the Euroline method may be recommended as an alternative routine clinical allergy diagnostic work up to determine sensitization profiles to timothy grass and birch pollen.

Fundings

The study was financed by grants from the Swedish state under the agreement between the Swedish government and the county councils, the ALF agreement (grant number OLL-842691).

Contributions

AS, SH, OH: conceptualization. AS, SH, OH, LB, MB: study design. AS, MB: analytical approach. LB: laboratory analysis. MB: data cleaning and analysis, draft. AS: supervision. All authors: interpretation of results, manuscript revision prior to submission.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgements

We express our thanks to all patients who were included in this study.

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Mohammad Hassan Bemanian[1], Ali Bassir[2], Kourosh Mohammadi[3], Mohammad Nabavi[1], Alireza Shafiei[4]

Anaphylaxis reaction to Samsum ant (*Pachycondyla sennaarensis*): a case series study

[1]Department of Allergy and Clinical Immunology, Rasool-E-Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
[2]Department of Radiology, Ronald Reagan-University of California Los Angeles Medical Center, David Geffen School of Medicine at UCLA, Los Angeles (CA), U.S.A.
[3]Department of Pediatrics, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[4]Division of Allergy and Clinical Immunology, Department of Pediatrics, Bahrami Hospital, Tehran University of Medical Sciences, Tehran, Iran

Key words
Anaphylaxis; ant venoms; insects allergy; hypersensitivity; insect bites and stings.

Corresponding authors
1) Alireza Shafiei
Bahrami Hospital
Tehran University of Medical Sciences
Kiaei Ave, Damavand Street
Tehran, Iran 1641744991
ORCID: 0000-0002-1478-7225
E-mail: ar.shafii@gmail.com
2) Ali Bassir
Ronald Reagan UCLA Medical Center
David Geffen School of Medicine at UCLA
10833 Le Conte Avenue
Los Angeles (CA) 90095, U.S.A.
ORCID: 0000-0001-8458-5525
E-mail: alibassir@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.246

Summary
Background. *Anaphylaxis is a life-threatening hypersensitivity reaction. The present study aimed to investigate the cases of anaphylaxis to ant stings in Iran to determine the characteristics of patients, geographical distribution and the type of ants that cause anaphylaxis.* ***Methods.*** *Patients with a history of anaphylaxis to ant sting underwent skin allergy test with extracted substance from Solenopsis invicta. Samples of ants were collected from the sites where each patient was bitten, and their species were identified by a medical entomologist.* ***Results.*** *Nineteen patients (mean age 26.2 years; range 4-48 years) were included in the study. Most patients (89.5%) were female. The lower limb was the most common site of the sting and most stings had occurred in the morning (31.6%) and evening (31.6%). Skin manifestations were the most common clinical symptoms (94.7%). Most cases of stings were observed in the Hormozgan province (89.5%) located in southern Iran. Sixteen patients had positive skin prick test for ant venom. All collected ants that caused anaphylaxis belonged to the Pachycondyla sennaarensis species.* ***Conclusions.*** *Ant sting anaphylaxis is not uncommon in Iran, especially in its southern regions. All cases of anaphylaxis in this study were due to Samsum ant sting (Pachycondyla sennaarensis), which is a species similar to the fire ant (Solenopsis invicta). Allergy skin testing with fire ant extract was positive and helpful in identifying Samsum ant allergy in all cases.*

Impact statement
This study is the first case series report from anaphylaxis to ant sting in Iran and all cases of anaphylaxis in this study were due to Samsum ant sting.

Introduction

Anaphylaxis is an acute systemic hypersensitivity reaction caused by the release of various mediators from mast cells and basophils and can be induced by various triggers, including foods, drugs, and stinging insects (1-3). Stinging insects of the Hymenoptera order can cause anaphylaxis. Systemic allergic reactions to insects' stings are reported by up to 3% of adults and about 1% of children have a medical history of severe reactions to insect sting (4). In a cross-sectional study of Bemanian *et al*., the frequency of anaphylactic reaction in adults and children was 169 and 46 cases per 100,000, respectively (5). There are three families of clinically significant Hymenoptera: the bees (honeybees, bumblebees), vespids (yellow jackets, hornets, wasps), and stinging ants. There are several species of ants that their stings can lead to anaphylaxis, including Formicidae such as fire ants (*Solenopsis invicta*), *Myrmecia* spp., *Pogonomyrmex* spp., and *Pachycondyla* spp. (6). Fire ants are responsible for most allergic reactions to ant stings in the United States (7). Jack jumper (*Myrmecia pilosula*) in Australia, *Pogonomyrmex* spp. in Canada and *Pachycondyla* spp. in Asia and the Middle East are the most common causes of ant stings hypersensitivity (8-10). In Africa and the Middle East, reported cases of ant sting anaphylaxis are almost always caused by *Pachycondyla* (*Brachyponera*) *sennaarensis* (10). There have been no reports of anaphylaxis to ant in Iran until now. In this study, we present the first report of anaphylaxis to ant in Iran over 10 years as a case series.

Materials and methods

This study was conducted at the Allergy Research Center of Rasool-E-Akram Hospital in Tehran, Iran. The study was conducted in full accordance with the principles outlined in the Helsinki Declaration of 1975. The study protocol was reviewed and approved by the Human Research Ethics Committee of Iran University of Medical Sciences (Approved Number: IR.IUMS.FMD.REC 1394.2272). All participants signed an informed consent form after being informed about the study protocol.

According to scattered reports of anaphylaxis to ant stings in Iran, after contacting Iranian allergists, they were asked to refer all cases of ant anaphylaxis from 2004 to 2014 to the Allergy Research Center of Rasool-E-Akram Hospital in Tehran and a private allergy clinic in Yazd. All patients who had a history of generalized systemic allergic reaction to ant and their history were compatible with clinical criteria for the diagnosis of anaphylaxis were included (11). Subjects were excluded from the study if investigation based on the patient's history indicated that no systemic reaction had occurred or that the ant sting was not the cause of the reaction. At study enrollment, patients' demographic and clinical characteristics, including their age, sex, time of the incident, geographical location, clinical manifestations of the reaction, any history of hypersensitivity to ant bites, medical history, and history of drug or alcohol use before the reactions were recorded. In addition, the records of patients at emergency department, where patients were admitted in case of hypersensitivity reactions, were reviewed and the data were integrated. The severity of the anaphylactic reaction for each individual was rated as mild, moderate, or severe according to the criteria published by Brown (12). This rating is based on the clinical manifestations of the anaphylactic reaction and the organs involved in the anaphylaxis. We asked a family member or friend of the participants who had no history of ant allergy to collect four samples of ants from each place where the patient reacted. The ants' specimens were identified by a medical entomologist regarding their unique entomologic phenotype. In addition, participants were asked to identify the ant responsible for their reaction based on the appearances of the ants.

All subjects underwent skin prick test with extracted substance from *Solenopsis invicta* (commercial solutions of Hollister-Stier Allergy Company, Chicago, USA). The tests were performed by an allergist according to the manufacturer's recommended protocol at the hospital. Patients with negative response in skin prick test underwent intradermal skin allergy test with ten times diluted extract. All tests were done with histamine chloride 10 mg/ml as positive and sodium chloride 0.9% as negative controls. The test response was read after 15 minutes. A wheal diameter caused by tested allergens more than 3 millimeter compared to the negative control was considered as a positive response and sensitization to that allergen.

Statistical analysis

Statistical analysis was performed using Stata statistical software (Stata 13, Stata Corp, Texas, USA). Descriptive data were expressed as mean (standard deviation) or median (range) for continuous variables and number (percent) for categorical variables, respectively. Data regarding features of sting, clinical manifestation, medical history and severity grading of anaphylaxis were further summarized in respective tables.

Results

Nineteen participants (mean age 26.2 years; range 4-48 years) met the inclusion criteria. The majority of subjects (17 out of 19) were female. Data related to occurred reactions after ant sting, such as reaction characteristics, geographic locations, and anatomical locations, are presented in **table I**. Most stings had occurred in the morning (31.6%) and evening (31.6%) (**table I**). Most cases of stings were observed in Hormozgan province located in the south of Iran (89.5%) and the capital of Hormozgan province in Bandar Abbas (47.4%) (**figures 1, 2**). The lower limb was the most common site to be bitten (**table I**). Each patient had an average history of 4.2 previous systemic reactions due to ant sting (range

2-10 times). Ant sting in most cases led to a systemic reaction (94.7%), and local reaction occurred only in 5.3% of patients. According to patients' medical records, 78.9% had a history of at least one atopic disease. Allergic rhinitis (73.3%) and asthma (15.8%) were the most common allergic diseases among the participants. In 31.6% of cases, subjects had a history of underlying disease, the most common of which was cancer. Family history of patients showed that 52.6% of subjects had a family history of allergic disorders. Among family members, the prevalence of allergies was higher in mothers (21.1%) and asthma was the most common allergic disease (21.1%). A summary of the participants' medical history is provided in **table I**.

The mean time interval between stings to the first clinical manifestation was 3.7 minutes (range 1-10 minutes). Information on the clinical manifestations after sting and its management are presented in **table II**. According to the records of patients admitted to the

Table I *- Characteristics of the study population.*

Characteristics	**n = 19, n (%)**
Gender	
Male	2 (10.5)
Female	17 (89.5)
Anatomical position of stings	
Foot	10 (52.6)
Hand	4 (21.1)
Trunk	2 (10.5)
Head and neck	3 (15.8)
Place of sting	
Inside	12 (63.2)
Outside	7 (36.8)
Time of sting	
Morning	6 (31.6)
Noon	3 (15.8)
Afternoon	6 (31.6)
Night	4 (21.1)
Personal history of atopy	
Asthma	3 (15.8)
Allergic rhinitis	14 (73.7)
Urticaria	2 (10.5)
Family history of atopy (based on disease)	
Asthma	4 (21.1)
Allergic rhinitis	3 (15.8)
Allergy to insect stings	3 (15.8)
Family history of atopy (based on affected person)	
Mother	4 (21.1)
Father	2 (10.5)
Sister	3 (15.8)
Brother	1 (5.3)
Drug history	
Anti-histamine and NSAIDs	3 (15.8)
History of underlying disease	
Cancer	2 (10.5)
Cardiovascular	1 (5.3)
Other diseases	3 (15.8)

Table II *- Characteristics of clinical reactions and management performed after ant stings in the study population.*

Characteristics	**n = 19, n (%)**
Affected organs	
Cutaneous	18 (94.7)
Respiratory	17 (89.5)
Cardiovascular	9 (47.7)
Neurological	7 (36.8)
Gastrointestinal	6 (31.6)
The severity of anaphylactic reactions	
Mild	1 (5.3)
Moderate	11 (57.9)
Severe	7 (36.8)
Post-sting actions	
Referred to the emergency	17 (89.5)
Self-treatment with antihistamine	2 (10.5)
Self-injecting epinephrine	
Having epinephrine	4 (21.1)
Use of epinephrine	0 (0)
Response to injected epinephrine in the Hospital	
Good response to the first dose	15 (88.2)
Required repeated-dose	2 (10.5)
Reasons for not participating in immunotherapy	
Uncertainty about treatment	1 (5.3)
Difficult commuting to the treatment center	4 (21.1)
Expensive treatment	6 (31.6)
Adverse reactions to immunotherapy	
No reaction	4 (21.1)
Local reaction	3 (15.8)
Anaphylaxis	1 (5.3)
Reaction to ant stings after starting immunotherapy	
No reaction	4 (21.1)
Mild local reaction	1 (5.3)
Anaphylaxis	1 (5.3)
No exposure to ant stings	2 (10.5)

Figure 1 *- The geographical locations of stings sites on the map.*

Google Maps. Ant bite map. Available at: https://www.google.com/maps/@29.0938586,58.027778,6.49z.

Figure 2 *- The geographical locations of sting sites on the map in a closer view.*

Google Maps. Ant bite map. Available at: https://www.google.com/maps/@27.6639202,57.2677472,7.49z.

emergency departments, skin manifestations were the most common clinical symptoms, which occurred in 94.7% of patients (**table II**). Pruritus, flushing, and dyspnea occurred in 89.5%, 73.3%, and 73.7% of subjects, respectively. None of the participants had cyanosis, abdominal cramp, bronchospasm, or incontinence (**figure 3**).

After the sting incident, patients were evaluated in terms of actions after the sting. Most cases (89.5%) referred to the emergency department and two patients had taken oral antihistamines themselves and did not go to the emergency department. At the time of the ant sting, only 21.2% of patients had epinephrine, but none of them used it at the time of the attack. Of the 15 patients referred to the emergency department, 88.2% responded well to the first dose of epinephrine and 11.8% required repeated epinephrine (**table II**). None of the patients needed cardiopulmonary resuscitation. The average length of stay in the emergency room was 2 hours (range 1 to 5 hours). Out of 15 patients referred to the emergency department, three patients (17.6%) needed to be hospitalized. After discharge, EpiPen (self-injecting epinephrine) was prescribed to only four patients and no action plan for anaphylaxis was given to any patient.

According to Brown's model for classifying the severity of anaphylactic reactions (12), moderate reactions were the most common anaphylactic reactions (57.9%). The results of grading the severity of anaphylactic reaction in participants are presented in **table II**.

Participants' allergy to ant venom was confirmed by skin allergy tests. Initially, all patients underwent skin prick test (SPT). The SPT results showed that 16 out of 19 patients have positive SPT for ant venom. The other three patients were then tested by intradermal test, and we found that they were also allergic to ant venom. Two patients showed a systemic reaction during skin tests and were treated immediately.

A medical entomologist evaluated the collected ant samples to determine the species of ants that caused the allergic reactions. He confirmed that all ant samples belonged to *Pachycondyla sennaarensis* species (**figure 4**).

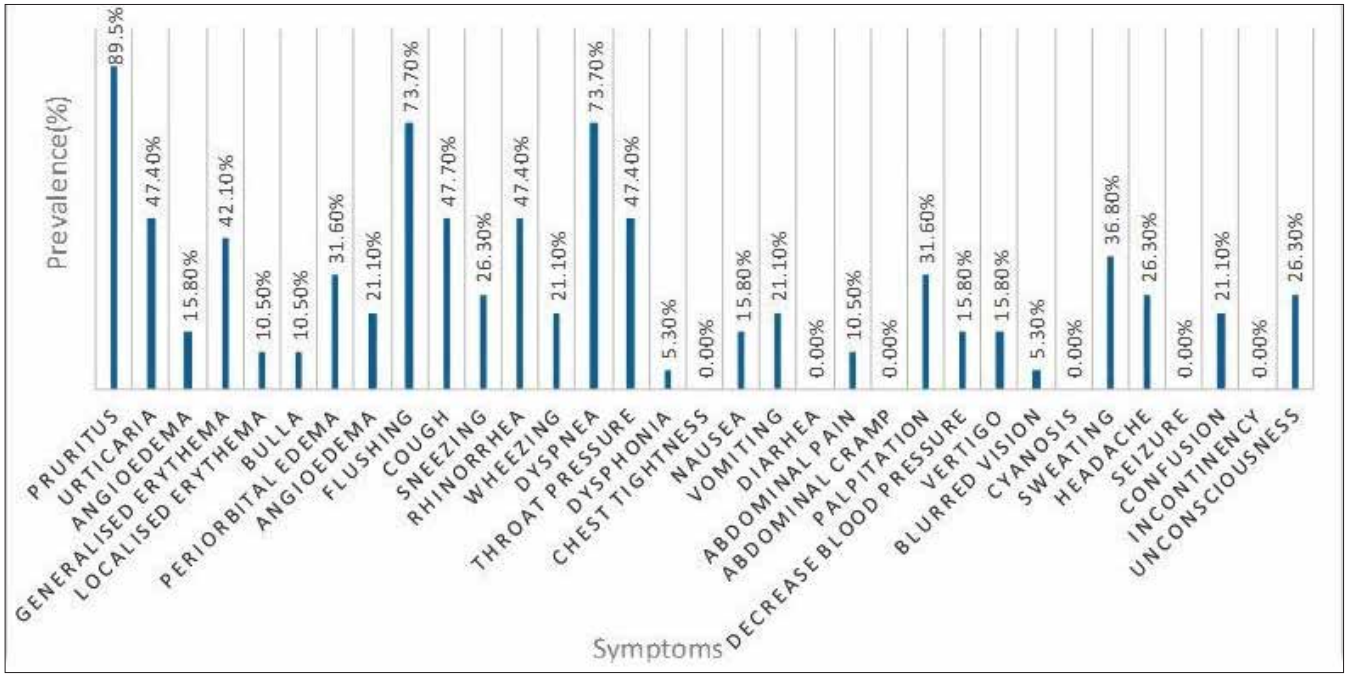
All patients were asked to receive immunotherapy and 42.1% accepted the proposed treatment. Patients stated that the most common reason for not participating in immunotherapy was the high cost of treatment (**table II**). Venom immunotherapy was performed with the GREER® (Allergenic extracts – Ant Fire, Solenopsis Invicta; Lenoir, United States of America). Of the eight patients who underwent immunotherapy, 50% had adverse reactions to immunotherapy. The most common complication was a local reaction at the injection site. Of the eight patients treated with immunotherapy, six were re-sting by ants, and only one developed anaphylaxis (**table II**).

Discussion

The prevalence of anaphylaxis in the US population is estimated at 1.6-5.1% (13). Anaphylaxis to insect stings is responsible for 20-30% of all anaphylaxis cases referred to emergency services (13, 14). It is estimated that about 0.4 to 0.8% of children and 2 to 3% of adults experience a systemic reaction to insect stings during their lifetime (15, 16). Anaphylaxis following insect stings is a major challenge for allergists. The mortality rate from anaphylaxis due to insects' sting is reported to be approximately 0.1 cases per million population (17).

Some ants are in the group of stinging insects. However, in a small number of ants, their stings cause allergic reactions (18). *Pachycondyla sennaarensis* (PS) is an ant species widely distributed throughout sub-Saharan Africa and the Middle East (19, 20). In some countries

Figure 3 *- Frequency of symptoms in patients.*



around the Persian Gulf, there have been reports of anaphylaxis due to the sting of PS (21, 22). In a case report from Saudi Arabia in 2009 by AlAnazi *et al*., they reported four patients with anaphylaxis due to PS (22). They described that PS ants, like fire ants, inject their venom with a sting and do not bite. In Iran, the presence of PS was first reported in 2004 by Akbarzadeh *et al*. in Sistan and Baluchestan (a province in southeastern Iran). They described the ant's morphology with a punctuated head and chest, a mandibular triangle, and seven teeth (23). In a study conducted by Paknia *et al*. In Iran, they found that PS was more prevalent in Iran, especially in the south (24). Most reported cases of ant stings in Iran were in Hormozgan province in southern Iran and most of them were from Bandar Abbas city in this province. By comparing the distribution of PS ants in Iran as mentioned and the distribution map of ant bites in this study (**figures 1, 2**), we understand that they are in the same areas. Khoobdel and his colleagues claim that shipping to southern countries around the Persian Gulf may be the cause of the PS entering Iran (25).

In a study by Nikbakht *et al*. in 2009, they claimed that there was no report of anaphylaxis following PS stings from Iran until that time. Based on their research in the biology and chemical diversity of PS abdominal glands, they explained the lack of anaphylaxis with low protein content in the abdominal glands of PS in Iran (26). Here, we report 19 cases of anaphylaxis following PS sting, which is the largest report on this issue from the Middle East. Most cases of anaphylaxis to the PS sting lived in southern Iran.

Figure 4 *- Photograph of Pachycondyla sennaarensis (black Samsum ant).*

The mean age of patients in our study was 26.2 years and most cases were female (17 out of 19 years). The mean age of patients in other studies of anaphylaxis to insect stings in Australia and Spain were 46 and 40 years, respectively (8, 27). Comparing the mean age of the population of Iran and other countries in which

the above studies have been performed (Iran = 28.3, Australia = 38.3 and Spain = 41.6) shows that the population of Iran is younger than Australia and Spain. It may be explained why patients' mean age in our study was lower than other studies. In most studies of anaphylaxis to insect stings, there was no significant difference in prevalence between men and women (12, 28-30). In our study, the prevalence of anaphylaxis to insect bites was significantly higher in women. It is difficult to explain the reason for this observation due to the small number of cases. In the present study, most stings occurred in the morning and evening. In a survey by Khoobdel *et al.* on the biological behavior of ants in the tropics and subtropics, they explained that ants leave their nests shortly before sunrise and work for several hours as the earth warms. They return to their nest, and if they are far away, they hide in the hole of the earth and resume their activity in the evening (25). This behavior of the ants may explain why most of the stings occurred in the morning and evening.

In current study, the lower limb was the most common anatomical site of the sting. In the study of Khoobdel *et al*. on ant bites in Abu-Musa Island, in the south of Iran, the lower limb was also the most stinging site (50.7%) (25). It is probably because the lower limbs are the most accessible part of the body for ants. Skin manifestations were the most common clinical manifestations in our study, followed by respiratory, cardiovascular, neurological, and gastrointestinal manifestations, respectively. In a survey conducted by Jirapongsananuruk *et al*. in Thailand on the characteristics of patients admitted with anaphylaxis, the most common manifestations were cutaneous (86%), respiratory (80%), cardiovascular (52%) and gastrointestinal (36%), respectively (29). In another study by Brown *et al*. on 1,149 patients with anaphylaxis in Australia, pruritus (73%), erythema (48%), angioedema (39%) and dyspnea (29%) were the most common symptoms (12). In our study, pruritus, dyspnea, and flushing were the most common symptoms. The order of common manifestations in our study seems to be almost the same as in Brown's and Jirapongsananuruk's studies. In addition, three patients (17%) of all our subjects required hospitalization, which is in line with the result of Jirapongsananuruk's study, where 12% of patients with anaphylaxis were hospitalized (29). On average, the patients had a history of four-time stings leading to anaphylaxis in their history. A study by Webb *et al.* on anaphylactic patients showed that more than half of the patients experienced more than three episodes of anaphylaxis (30).

In our study, 78.9% of patients had a history of atopic disorder. Allergic rhinitis, asthma and urticaria were the most common diseases in 73.7%, 15.8% and 10.5%, respectively. In the study of Jirapongsananuruk *et al*. in anaphylactic patients, 52% of cases had a history of atopy and the most common atopic diseases were asthma (26%), allergic rhinitis (20%) and drug allergies (16%) (23). In another study by Web *et al*., anaphylaxis was more common in atopic patients, in which 54% of cases with exercise-induced anaphylaxis and 50% of patients with food-induced anaphylaxis had a history of atopic disorders (30). In a study by González-Pérez *et al*., the incidence of anaphylaxis was higher in patients with asthma than in patients without asthma, and this rate was higher in severe asthma (31). In a patient with asthma, special attention should be paid to controlling respiratory manifestations. Several studies have shown that inadequate treatment of asthma is a significant risk factor for severe anaphylaxis (32, 33).

Alcohol and drug use before anaphylaxis may affect the severity of attacks (34). In our study, no one had consumed alcohol, and 15.8% of them had taken drugs such as aspirin, ibuprofen, and cetirizine. In the study of Wölbing *et al.* regarding the effect of cofactors on anaphylaxis, they found that the use of alcohol and drugs (such as NSAIDs) can cause a more severe reaction with a small amount of allergen and reduce the interval between exposure to allergens and anaphylaxis (35).

Regarding the underlying disease, 31.6% of the evaluated patients had underlying disease, including cancer (10.5%) (breast and uterine cancer), cardiovascular diseases (5.3%), thalassemia (5.3%), favism (5.3%) and diabetes (5.3%). In the Jirapongsananuruk's study of anaphylactic patients, 31% of them had an underlying disease, which the most common disease were: cancer (11%), cardiovascular diseases (7%) and neuromuscular diseases (6%) (29). Cancer and cardiovascular disease appear to be the most common underlying diseases in patients with anaphylaxis.

We asked patients about the history of ant stings without anaphylaxis, and only 5.3% reported having a history of ant stings without anaphylaxis. Given that in IgE-mediated systemic reactions to Hymenoptera sting, the previous sting is necessary for sensitization to venom proteins, and systemic reactions occur only with repeated stings after previous sensitization. We suspect that the reason why most patients, contrary to expectations, did not report a history of ant sting before anaphylaxis may be that patients had mild and non-systematic reactions to the initial stings and forgot the mild reactions due to its low severity and only recalled severe systemic reactions to ant stings. About family history of allergic disease in the evaluated patients, 52.6% of them had a family history of allergic diseases. It was more common in the mother of patients (21.1%) and the most common disease was asthma (21.1%). In our cases, allergic rhinitis was the most common atopic disease in their history and asthma was the most common atopic disease in their family. In a study by Sheffer *et al*. about exercise-induced anaphylaxis, they found that all patients had a family history of allergic rhinitis and 13% of them had a family history of asthma (36).

Skin allergy tests were performed on all our patients and all of them had positive skin tests. We used *Solenopsis invicta* extract instead of *Pachycondyla sennaarensis* extract in the skin tests as PS extract was unavailable. Although the PS ant sting was re-

sponsible for anaphylaxis in all cases in our study, skin tests with *Solenopsis invicta* extract were positive in all patients. The similarity between the materials extracted from *S. invitca* and PS seems to lead to a positive result in skin testing.

Two patients showed anaphylactic manifestations during skin allergy test and were treated immediately. This indicates that although the amount of substance used in the skin allergy test is minimal, it may lead to anaphylaxis and a well-equipped medical team should be present in the test room. In a study on the risk of anaphylaxis during skin allergy test by Liccardi *et al.*, they found that the risk of anaphylaxis during skin prick test was less than 0.02% and with intradermal skin test was higher, and in some cases, intradermal skin test could be fatal. Given this risk, they recommended that intradermal testing should not be the first option for assessing allergies (37). The high rate of anaphylaxis in the skin allergy test in our study seems to be due to selected patients, all of whom have a history of ant sting anaphylaxis.

According to the classification system of severity of anaphylactic reactions presented by Brown *et al.* (12), we found that most anaphylactic reactions in our patients were moderate (57.9%) followed by severe (36.8%) and mild (3.5%). In Brown's study, they analyzed 1,149 patients with anaphylaxis. Most of anaphylactic reactions were severe (68%), followed by moderate (42%) and mild (15%). When they focused on patients with anaphylaxis due to insects stings, they found that most reactions were moderate (47%), then mild (35%) and severe (16%). It seems that in patients with anaphylaxis due to insect bites, most patients show a moderate reaction.

It should be noted that in the present study, Iranian allergists were asked to refer patients with a history of ant sting anaphylaxis. It may reduce the actual number of cases of ant sting anaphylaxis due to the probable cases that have not been referred to an allergist. The use of patients' previous history may also affect our data recall biased. Furthermore, another limitation of the present study is that the Samsum ant extract was not available to us for skin testing. Instead, we used fire ant extract. Although all of our cases had a positive skin test to fire ants (probably due to the similarity of the allergens in the extracts), it would be better to use Samsum ant extract for skin testing. This report is the first case series of anaphylaxis to ants in Iran and the largest report in this field in the Middle East. The present report shows that ant sting anaphylaxis is not uncommon in these areas. Although fire ants (*Solenopsis invicta*) are the most common cause of ant stings in Europe and the United States, all cases of anaphylaxis in this study were due to the sting of Samsum ant (*Pachycondyla sennaarensis*), a species similar to the fire ant, that is found in the south of Iran, the Middle East and North Africa. Allergy skin testing with fire ant extract was positive and helpful in detecting Samsum ant allergies. In cases where Samsum ant extract is not available, fire ant extract may be a good alternative to Samsam ant skin test.

Fundings

None.

Contributions

MHB: study design. AB, MHB: data collection. AB, AS, MHB: data interpretation. AB, AS: writing – original draft. AS, KM, MN, MHB, AB: manuscript revision and approval. KM: referred most cases and few cases referred by MN.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

The authors would like to appreciate the valuable cooperation of all Allergists and Clinical Immunologist of Iran in referring patients, Dr. Kamran Akbarzadeh (Medical Entomologist, Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran) for his invaluable assistance in identifying the ants' specimens and the staff of Rasool-E-Akram Hospital Allergy Research Center and the private allergy clinic in Yazd.

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ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 55, N.2, 86-93, 2023

Joana Barradas Lopes, Cátia Santa, Cristina Valente, Ana Rita Presa, Maria João Sousa, Ana Reis Ferreira

Allergy to lipid transfer proteins (LTP) in a pediatric population

Department of Allergy and Clinical Immunology, Centro Hospitalar Vila Nova de Gaia/Espinho, EPE, Vila Nova de Gaia, Portugal

Key words

LTP; food allergy; fruit; tree nuts; children.

Corresponding author
Joana Barradas Lopes
Department of Allergy and Clinical Immunology
Centro Hospitalar Vila Nova de Gaia/Espinho
Rua Arquiteto Januário Godinho 37
4100-285 Porto, Portugal
ORCID: 0000-0002-9798-5429
E-mail: joanabarradaslopes@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.229

Summary

Background. *Lipid transfer proteins (LTP) are considered important plant-food allergens in the Mediterranean area, but little is known about LTP allergy in pediatric age. Our aim was to characterize LTP allergy in children.* ***Methods.*** *We reviewed the clinical data from all children evaluated in our department with LTP allergy. From the 76 patients with LTP allergy, 26 children were included, 50% female, median age 10 years (1-17). Symptoms included urticaria in 58% (n = 15), anaphylaxis in 46% (n = 12) and OAS in 42% (n = 11).* ***Results.*** *Multiple reactions with different foods occurred in 69%. Cofactors were reported in 27% (n = 7). All patients had positive SPT to peach LTP extract and sIgE Pru p 3. No association between the occurrence of severe reactions and sIgE to Pru p 3 (p = 0.462), sIgE to Cor a 8 (p = 0.896), SPT to peach LTP extract (p = 0.846) or the number of positive SPT to fruits/tree nuts (p = 0.972; p = 0.676) was found. Ninety-two percent of the patients tolerated fruits from Rosacea family without peel. Twelve percent reported reactions to new LTP containing foods during follow-up. LTP allergy can occur since early childhood.* ***Conclusions.*** *Since anaphylaxis is common and cofactors act as severity enhancers, it is fundamental to recognize LTP allergy in children. Currently available diagnostic tests (SPT and sIgE) cannot accurately predict food tolerance or anticipate reaction severity.*

Impact statement

LTP allergy can occur since early childhood and be severe, with food tolerance and severity of reactions being unpredictable making the follow-up of these patients essential.

Introduction

Non-specific lipid transfer proteins (LTP) are panallergens present in plant-foods, being the most relevant allergens of Rosacea family fruits in the Mediterranean area (1, 2).

These ubiquitous proteins are highly conserved and widely distributed in the plant kingdom, sharing a moderate-to-high homologous molecular structure, which put the patients allergic to LTP at risk of developing allergic reactions after the ingestion of an array of botanically unrelated foods, including fruits, tree nuts, seeds, vegetables and cereals (1, 3-7).

Despite the fact that milk and egg proteins are the main causes of food allergy in childhood, fruits and vegetables have been recognized as emergent allergens in pediatrics (8).

Since LTP may cause severe systemic reactions, it is essential to better understand this allergy in childhood.

Even though multiple studies on LTP have been published in the last years, so far, not much is known about LTP allergy in

children, especially regarding daily practice, since only a few case series focused on pediatric ages have been published (9-13). A recent study has shown that fresh fruits are the 5th cause of anaphylaxis in children, with tree nuts being the 2nd, both potentially caused by LTP in this region (14).
Our aim was to characterize a series of children with allergy to LTP, in order to better understand its characteristics.

Materials and methods

We performed a retrospective analysis of medical records from patients under 18 years-old with confirmed LTP allergy (2013-2019). Diagnosis was established based on a convincing history of immediate allergic reactions to plant-foods (*i.e.*, repeated symptoms to LTP containing foods on several occasions) supported by positive skin prick tests (SPT) (defined as the mean diameter of the wheal ≥ 3 mm than negative control (15)) to LTP extract (Roxall, Bilbao, Spain) and positive specific IgE (sIgE) to LTP allergens (Pru p 3, Cor a 8 and/or Jug r 3) determined by ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden; cut-off: ≥ 0.35 kUA/L) and/or ImmunoCAP™ ISAC microarray (ThermoFisher, Uppsala, Sweden; cut-off: ≥ 0.3 ISAC Standardised Unit, ISU, as per manufacturer's recommendation). sIgE to Pru p 3 were determined in all children. Since sIgE to Cor a 8 and Jug r 3 were not available in our center from the beginning of the study, they were not determined in all. ImmunoCAP™ ISAC microarray 112 (ThermoFisher, Uppsala, Sweden) was performed in 9 children, providing information about the referred LTP allergens.
SPT with airborne allergens were performed in all patients. The SPT was conducted using a standard allergen extract panel and comprised histamine and saline respectively as positive and negative controls. For children under 6 years-old, the panel tested included: *Dermatophagoides pteronyssinus*; *Dermatophagoides farinae*; *Lepidoglyphus destructor*; cat; dog; olive tree; grass pollen mix; parietaria and *Alternaria alternata*. For children with ≥ 6 years-old, the panel tested included: *Dermatophagoides pteronyssinus*; *Dermatophagoides farinae*; *Lepidoglyphus destructor*; *Tyrophagus putrescentiae*; cat; dog; birch; plane tree; olive tree; grass pollen mix; *Cynodon*; mugwort; parietaria; plantago; ambrosia; *Cladosporium*; *Alternaria alternata*; *Aspergillus fumigatus*.
The decision of performing SPT to other food extracts available at our center (24 fruits, 8 tree nuts, peanut, soy and 4 seeds extracts) was made considering the child's age and collaboration, regardless of food tolerance.
The co-sensitization to other relevant proteins in fruit and tree nuts allergy, namely profilins, PR-10 and storage proteins, was evaluated, according to the severity of the reaction, the food trigger and the laboratory availability. Co-sensitization to profilins was evaluated in all patients, by SPT to profilin (n = 19) and/or ImmunoCAP™ ISAC microarray 112 (n = 9). Among the PR-10 family, Bet v 1 was the protein tested in all patients, by ImmunoCAP (n = 17) or ImmunoCAP™ ISAC microarray 112 (n = 9). Storage proteins were tested in 14 patients, according to the suspected food. In case of doubt (*e.g.*, mixed unidentified tree nuts), all the three families were tested (Ara h 1, Ara h 2 and Ara h 3).
Clinical manifestations were classified as local (oral allergy syndrome (OAS), contact urticaria) or systemic (urticaria, anaphylaxis). Severe reaction was defined as the occurrence of anaphylaxis. The presence of cofactors (exercise and NSAIDs) and food tolerance were investigated. Food tolerance was defined as no reactivity to food in patients' usual diet.

Informed Consent and Ethics committee approval

This project was conducted with the approval of Ethics Committee of the Research in our center. Informed consent was obtained from the legal responsible of each child.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 24 software (SPSS Inc, Chicago, Illinois). Descriptive statistics was performed. Categorical variables are presented as frequencies and percentages, and continuous variables as mean ± standard deviation (SD) or median with minimum and maximum values in brackets. Spearman's correlation, t-student test and Mann-Whitney tests were used. A 2-tailed P-value < 0.05 was considered statistically significant.

Results

Twenty-six children were selected from a universe of 281 followed in our Department for suspected food allergy; 50% were female, median age of first symptoms 10 (1-17) years-old. Age at first symptoms was 0 to 2 years-old (n = 3), 3 to 5 years-old (n = 5), 6 to 11 years-old (n = 6) and 12 to 17 years-old (n = 12). The median time for diagnosis after the initial episode was of 4 (0-8) years. Sixty-one percent were atopic, 58% had pollen sensitization, 54% rhinitis, 27% asthma, 19% atopic dermatitis, 1 patient had another food allergy (cow's milk) and 1 patient had eosinophilic esophagitis. The characteristics of the children included are detailed in **table I**. Different food triggers were identified, with 69% of patients reporting reactions to more than one food. Fruits were involved in 69% (n = 18), tree nuts in 50% (n = 13), peanut in 8% (n = 2) and sesame in 4% (n = 1). Peach was the most frequent trigger (62%, n = 16). Symptoms both with fruits and tree nuts occurred in 27% (n = 7).
Fifty eight percent (n = 15) had exclusively systemic symptoms, 15% (n = 4) exclusively local symptoms, and 27% (n = 7) had both. Symptoms included urticaria in 58% (n = 15), anaphylaxis in 46% (n = 12) and OAS in 42% (n = 11) of the patients. Demographic and clinical differences between patients reporting reactions exclusively to either fruits or tree nuts/peanut/seed

were evaluated. Patients exclusively allergic to fruits were 70% female, median age of first symptoms 4 (1-6) years and anaphylaxis occurred in 36%. Patients exclusively allergic to tree nuts/peanut/seed were 60% male, median age of first symptoms 12 (2-17) years and anaphylaxis occurred in 53%. Differences were also evaluated between patients allergic to either one food or multiple foods. Patients allergic to only one food were 54% male, median age of first symptoms 9 years (1-17) and anaphylaxis occurred in 39%. Patients allergic to multiple foods were 53% female, median age of first symptoms was 11 (1-17) years and anaphylaxis occurred in 54%. There were no statistical differences between the different groups.

Cofactors were involved in 27% (n = 7) of the patients: exercise in all and NSAIDs in 1 patient. In 2 patients cofactors were essential to the occurrence of reactions (anaphylaxis); in 5 patients the cofactors elicited more severe reactions (anaphylaxis in patients with urticaria and/or OAS (n = 1), urticaria in patients with OAS (n = 4)).

Regarding SPT, patients had a mean wheal of 7.1 mm (SD 3.81) to peach LTP, with a different spectrum of positivity to fruits and tree nuts, as detailed in **table II**. Thirty-eight percent (n = 10) tolerated fruits/tree nuts for which SPT were positive.

Results were concordant for both sIgE determination methods (ImmunoCAP™ and ImmunoCAP™ ISAC microarray 112). sIgE to Pru p 3 was positive in all patients (26/26), sIgE to Cor a 8 in 35% (8/23) and sIgE to Jug r 3 in 73% (8/11), regardless of the trigger (fruits/tree nuts) (**table III**).

Since sIgE to Cor a 8 and sIgE to Jug r 3 are proteins from tree nuts sources (hazelnut and walnut, respectively), the presence of sIgE to these allergens were evaluated in children with tree nuts as food triggers: sIgE to Cor a 8 was positive in 38% (5/13) and sIgE to Jug r 3 in 67% (4/6).

Co-sensitization to other panallergens was documented in 15% (n = 4): 2 patients to profilins (positive profilin in SPT) and 2 to PR-10 (Bet v 1). A broad spectrum of clinical manifestations occurred in these patients, ranging from OAS to anaphylaxis. Sensitization to storage proteins was not found.

A broad spectrum of fruit tolerance was found, with 92% of the patients showing tolerance to at least one fruit from Rosacea family without peel. Apple tolerance (a staple food in Portuguese diet) was present in 50%, unknown in 27% and absent in 23%. SPT to apple were positive in 4 (21%) patients (none of them tolerated the apple).

During follow-up, 12% (n = 3) patients reported reactions to new LTP containing foods, with different timings since the occurrence of the first reaction (< 1 year, 2 years, 8 years).

There was no association between the occurrence of severe reactions and pollen sensitization, comorbidities (rhinitis, asthma, atopic dermatitis), type of trigger (fruits/tree nuts), number of food triggers, mean wheal of SPT to peach LTP, the number of positive SPT to fruits or tree nuts or ImmunoCAP™ determination of sIgE to Pru p 3 or sIgE to Cor a 8 (**tables IV** and **V**).

Discussion

We present a pediatric series with documented LTP allergy, focusing on it in clinical practice. Considering all children referred to our department with suspected food allergy, 9% of them were diagnosed with LTP allergy, a low but not irrelevant percentage.

In our study, LTP allergy seems to be similar in terms of frequency between females and males, with more than a half reporting the first reaction before age 12. Twelve percent had their first reaction before age 3, which reinforces the importance of considering this diagnosis in infants and toddlers, as suggested by other authors (16).

Since LTP are widespread in the plant kingdom, it is not surprising that most children had more than one food trigger. Food triggers in LTP allergy are ubiquitous but probably less considered by the general population, since they are not "classical" allergens in children. This underestimation can make their recognition harder when reactions occur, explaining the delay in diagnosis verified in our study.

Clinical manifestations and severity of LTP hypersensitivity varied in our children, as described for adults (1-4, 9, 17). It is important to highlight that, although urticaria was the most frequent symptom, severe reactions were also common, with anaphylaxis occurring in almost half of the patients. Cofactors were present in more than one quarter of the children and the majority had more severe reactions in their presence; in some children, cofactors were essential for reactions to occur and were associated with anaphylaxis. This evidence supports the importance of cofactors as severe reaction inductors in LTP allergic children, as in adults (18-20).

The presence of risk factors for severe reactions was investigated. No associations were found with pollen sensitization, comorbidities, types/number of food triggers and co-sensitization, mean wheal of SPT to LTP extract, level of ImmunoCAP™ sIgE either to Pru p 3 or Cor a 8, the latest in agreement to November *et al.* (9). Other authors reported different results in adults, establishing an association between higher levels of IgE to Pru p 3 and systemic reactions with fruits from Rosacea family (21).

Co-sensitization to profilins and PR-10 was found in a low number of patients, with a diverse spectrum of clinical presentations, raising doubts about their clinical relevance. On the other hand, co-sensitization to storage proteins was not found, despite the fact that tree nuts were involved as exclusive triggers in one third of the patients, supporting the clinical relevance of LTP sensitization.

No association was found between tolerance to fruits from Rosacea family, mean wheal SPT with peach LTP extract, number of positive SPT to fruits and level of sIgE to Pru p 3. It is important to highlight that SPT to food extracts were not reliable methods to confirm clinical reactivity, since 38% of children had positive SPT to foods subsequently tolerated.

Food avoidance is the mainstay of treatment for LTP allergy and should be guided by clinical reactivity and not sensitization. As proposed by Asero *et al.* (3), all children were prompted to main-

Table I *- Characteristics of the children with LTP allergy included.*

	Age	Sex	Pollen sensitization	Comorbidities	Type of reaction	Food triggers	Tolerance to apple	Tolerance to fruits from Rosacea family	Cofactors
1	1	Male	No	Food allergy (cow's milk)	Urticaria	Peach	Yes	Only peeled	No
2	1	Female	No	Atopic dermatitis	Urticaria	Peach, nectarine, pomegranate	Yes	Only peeled	No
3	2	Female	Yes (mugwort, olive tree)	Rhinitis	Urticaria	Pine nut	Yes	Yes	No
4	3	Female	No	-	OAS, urticaria	Peach, pear, apple	No	Only peeled	No
5	3	Female	Yes (grass pollen, plane tree, mugwort, olive tree, birch)	Rhinitis	Urticaria, anaphylaxis,	Peach, apricot, apple, plum	No	Unknown	No
6	4	Male	Yes (grass pollen, plane tree, mugwort)	Asthma, rhinitis, atopic dermatitis	OAS	Peanut	Yes	Yes	No
7	5	Female	No	-	Urticaria, anaphylaxis	Peach, cherry	Yes	Only peeled	No
8	5	Male	No	Asthma, rhinitis	OAS, urticaria	Mixed unidentified tree nuts	Yes	Yes	Yes (exercise)
9	6	Female	Yes (grass pollen, plane tree)	Asthma, rhinitis	Anaphylaxis	Peach	Yes	Only peeled	No
10	6	Male	Yes (olive tree)	Rhinitis	Anaphylaxis	Apple, almond	No	Only peeled	No
11	7	Female	Yes (grass pollen, olive tree, birch)	Rhinitis	OAS, urticaria, anaphylaxis	Peach, kiwi, apple, almond	No	Only peeled	No
12	7	Male	No	-	OAS, anaphylaxis	Kiwi, pear, walnut	Yes	Yes	Yes (exercise)
13	9	Female	Yes (grass pollen, olive tree, birch)	Atopic dermatitis	OAS	Peach	Unknown	Only peeled	No
14	11	Male	Yes (grass pollen, plane tree, birch)	Asthma, rhinitis, atopic dermatitis	Urticaria	Mixed unidentified tree nuts	Yes	Yes	No
15	12	Female	No	-	Urticaria	Peach, mixed unidentified tree nuts	Unknown	Only peeled	No
16	12	Male	Yes (grass pollen, plane tree	Asthma, rhinitis	OAS	Peach, pear, walnut	Unknown	Only peeled	No
17	12	Male	No	-	Urticaria	Peach, apple, pear, plum, walnut, hazelnut, peanut, pistachio	No	No	No
18	13	Female	Yes (grass pollen)	-	OAS, anaphylaxis	Peach, apple, walnut, hazelnut	No	Only peeled	No

	Age	Sex	Pollen sensitization	Comorbidities	Type of reaction	Food triggers	Tolerance to apple	Tolerance to fruits from Rosacea family	Cofactors
19	15	Male	No	-	Anaphylaxis	Walnut	Unknown	Yes	Yes (exercise)
20	15	Male	No	-	Anaphylaxis	Sesame seeds	Unknown	Yes	Yes (exercise)
21	16	Male	Yes (grass pollen, mugwort)	Rhinitis	Urticaria, anaphylaxis	Peach	Yes	Yes	No
22	16	Male	No	-	Urticaria	Peach, apricot	Unknown	Yes	Yes (exercise)
23	16	Female	Yes (plane tree)	Rhinitis	OAS, urticaria, anaphylaxis	Peach, apricot, plum, pomegranate	Yes	Yes	Yes (exercise, NSAIDs)
24	16	Female	Yes (grass pollen, plane tree, mugwort, birch)	Asthma, rhinitis, eosinophylic esophagitis	OAS	Mixed unidentified tree nuts	Unknown	Yes	No
25	17	Male	Yes (grass pollen, plane tree, mugwort)	Asthma, rhinitis	OAS, urticaria	Peach, kiwi, cherry	Yes	Yes	Yes (exercise)
26	17	Female	Yes (mugwort)	Rhinitis	Anaphylaxis	Mixed unidentified tree nuts	Yes	Only peeled	No

tain the ingestion of tolerated foods without peel and minding the presence of cofactors. The purpose of this approach was the maintenance of a natural tolerance and the ingestion of important nutritious foods, as fruit and vegetables. In our sample, most children tolerated fruits from Rosacea family without peel, as expected since LTP are present mainly in the fruit peel (22, 23). Awareness of possible accidental-allergic reactions and the ability to correctly identify and adequately treat them is of extreme importance in these patients; children and their caregivers should be exhaustively educated about potential elicitors, timely reaction recognition, adequate treatment, and the role of cofactors in LTP allergy. Adrenaline auto-injectors were prescribed in all children with systemic reactions to LTP containing foods with or without cofactors and postponed in those who had only local reactions.

Twelve percent of children reported reactions to new LTP containing foods, with different timing considering the occurrence of first reaction. This should alert clinicians that LTP allergy may progress in number of eliciting foods.

We would like to highlight some aspects considered in our work that may not be consensual. We established LTP allergy diagnosis based on a convincing clinical history of immediate allergic reactions to plant-foods, defined as repeated symptoms to LTP containing foods on several occasions, supported by positive SPT to peach LTP extract and positive sIgE to LTP allergens (Pru p 3, Cor a 8 and/or Jug r 3).

We acknowledge that, based on the current evidence, doubts exist about the role of component resolved diagnosis when it comes to distinguish allergy from sensitization and possible food tolerances (16). However, we considered that the presence of a convincing clinical history, with reproducible reaction not or poorly explained by other plant food allergens, were the key in distinguishing sensitization from allergy.

Based on these considerations, we assumed that the combination of clinical history, results from *in vivo* and *in vitro* tests, in the absence of other plant food-allergens that could explain clinical manifestations, were enough to establish the diagnosis of LTP allergy and food challenges were protracted.

Nevertheless, as mentioned before, in a low number of patients, co-sensitization to profilins and PR-10 was found, raising doubts about the role of each allergen and their clinical relevance. We decided to assume a diagnosis of LTP allergy also in these patients, since all of them had systemic reactions, more usually associated in our country to LTP. However, we recognize that these interpretations can be a limitation in our study.

The retrospective nature of our study and limited number of patients are also limitations, reinforcing the importance of more studies in this area.

In conclusion, allergy to LTP can occur since childhood, even before school-age. Clinical manifestations of LTP allergy may vary, but the occurrence of anaphylaxis is common,

Table II *- Determination and characterization of all SPT performed.*

SPT	n performed	n positive	Mean wheal of positive SPT (mm)
Fresh fruit	389	89	-
LTP peach	26	26 (100%)	7.1 (SD 3.81)
Peach	21	17 (81%)	4.8 (SD 1.73)
Cherry	18	9 (50%)	4.2 (SD 1.92)
Apricot	17	8 (47%)	4.2 (SD 1.92)
Plum	17	7 (41%)	4.2 (SD 1.18)
Pomegranate	17	6 (35%)	4.2 (SD 0.93)
Grape	17	6 (35%)	4.0 (SD 0.74)
Kiwi	19	6 (32%)	3.4 (SD 0.66)
Fig	17	4 (24%)	4.0 (SD 1.68)
Apple	19	4 (21%)	4.5 (SD 0.71)
Strawberry	17	3 (18%)	3.5 (SD 0.87)
Loquat	17	3 (18%)	6.2 (SD 5.05)
Melon	19	3 (16%)	3,8 (SD 0.76)
Pineapple	17	2 (12%)	4.7 (SD 1.68)
Papaya	17	2 (12%)	4.0 (SD 0.71)
Pear	19	2 (11%)	4.0 (SD 1.41)
Mango	17	1 (6%)	-
Passion fruit	17	1 (6%)	-
Orange	17	1 (6%)	-
Watermelon	17	1 (6%)	-
Persimmon	17	1 (6%)	-
Avocado	17	1 (6%)	-
Banana	19	1 (5%)	-
Tree nuts/Legumes	153	56	
Hazelnut	20	12 (60%)	5.5 (SD 2.24)
Walnut	20	12 (60%)	5.1 (SD 2.66)
Almond	20	8 (40%)	3.6 (SD 0.82)
Peanut	20	7 (35%)	4.5 (SD 2.18)
Soy	19	6 (32%)	4.3 (SD 1.97)
Pine nut	18	5 (28%)	5.6 (SD 2.7)
Pistachio	18	3 (17%)	5.2 (SD 0.76)
Cashew	18	3 (17%)	3.5 (SD 0.5)

forcing it to be recognized as a potentially severe allergy in pediatrics. Cofactors may be essential to reaction occurrence and relate to more severe occurrences. No other risk factors to severe reactions were documented in our study. SPT to food extracts were not a reliable method to confirm clinical reactivity. Food avoidance is the mainstay of treatment however the ingestion of tolerated foods without peel should be maintained. More than 10% of the patients had subsequent reactions with new LTP-containing foods, reinforcing that follow-up is essential.

Table III *- Determination and characterization of sIgE to LTP determined both by ImmunoCAP™ and ImmunoCAP™ ISAC microarray.*

sIgE	n tested	n positive per patient and method	Median (KUa/L; ISU)
Pru p 3 (peach)	26	26	
ImmunoCAP™	19	19	2.7 (0.5-38.7)
ImmunoCAP™ ISAC microarray	9	9	1.6 (1.0-19.0)
Cor a 8 (hazelnut)	23	8	
ImmunoCAP™	14	6	0.1 (0.0-8.3)
ImmunoCAP™ ISAC microarray	9	5	0.7 (0.0-4.0)
Jug r 3 (nut)	11	8	
ImmunoCAP™	2	1	0.2 (0.0-0.4)
ImmunoCAP™ ISAC microarray	9	8	1.0 (0.0-10.0)
Ara h 9 (peanut)		6	
ImmunoCAP™ ISAC microarray	9	6	0.9 (0.0-8.0)
Tri a 14 (wheat)		1	
ImmunoCAP™ ISAC microarray	9	1	0.0 (0.0-1.0)

Table IV *- Statistical analysis evaluating a possible role of some continuous variables with severe reactions.*

Variables	Severe reactions (Mean Rank)		U	P-value
	Yes	No		
SPT with peach LTP extract	7.29	7.00	-	0.846
Number of positive SPT with fruits	4.75	4.82	-	0.972
Number of positive SPT with tree nuts	2.29	2.70	-	0.676
sIgE to Pru p 3	9.10	11.00	36	0.462
sIgE to Cor a 8	7.33	7.63	23	0.896

Units: sIgE (KUa/L); SPT (mm).

Table V *- Statistic analyses evaluating a possible role of some categorical variables with severe reactions.*

Variables	Severe reactions (N)		P-value
	Yes	No	
Pollen sensitization	7	8	1.000
Comorbidities			
Rhinitis	7	7	0.713
Asthma	1	6	0.081
Atopic dermatitis	0	4	0.100
Type of trigger			
Fruits	4	7	0.453
Tree nuts	3	5	0.683
Number of triggers (1 *vs* several)	5	8	0.695
Co-sensitization to profilins	2	0	0.203
Co-sensitization to PR-10	2	0	0.203

Fundings

None.

Contributions

JBL, ARF: study design. CV, ARP, MJS: data collection. CS: statistical analysis. JBL: writing – original draft; ARF: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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Natalia Pereverzina[1,*], Anastasiia Allenova[2,*], Elizaveta Gribaleva[3], Daniel Munblit[4-6], Dayana Skander[3], Pavel Kolkhir[2,7]

Restrictions related to COVID-19 can negatively affect Russian patients with chronic spontaneous urticaria

[1]Central State Medical Academy of the Administrative Department of the President of Russia, Department of Dermatology and Cosmetology, Moscow, Russia
[2]Laboratory of Immune-Mediated Skin Diseases, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation
[3]I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation
[4]Department of Paediatrics and Paediatric Infectious Diseases, Institute of Child's Health, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia
[5]Inflammation, Repair and Development Section, National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London, U.K.
[6]Research and Clinical Center for Neuropsychiatry, Moscow, Russia
[7]Department of Dermatology and Allergy, Charité - Universitätsmedizin Berlin, Berlin, Germany
*The authors contributed equally to this work

Key words

Chronic spontaneous urticaria; coronavirus; SARS-CoV-2; COVID-19; restrictions; telemedicine; digital medicine service; pandemic impact.

Corresponding author
Pavel Kolkhir
Laboratory of Immune-Mediated Skin Diseases
I.M. Sechenov First Moscow State Medical University (Sechenov University)
4/2, B. Pirogovskaya Street
119991 Moscow, Russia
ORCID: 0000-0001-5380-8132
E-mail: pavel.kolkhir@yandex.ru

Doi
10.23822/EurAnnACI.1764-1489.226

To the Editor,

chronic spontaneous urticaria (CSU) is a mast cell–driven skin disease characterized by the recurrence of transient wheals, angioedema, or both for more than 6 weeks without specific external stimuli. Multiple factors can influence the course of the disease and management of CSU including underlying conditions and triggers (1), for example respiratory tract infections. Recently, CSU has been discussed in the context of coronavirus disease (COVID-19) caused by severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) (2, 3). COVID-19 is characterized by significant morbidity and mortality especially in patients with chronic diseases (4). A recent study reported that COVID-19 results in exacerbation of chronic urticaria in one of three patients, mostly in patients with severe COVID-19 (5-7). Although one study from Turkey showed that patients with chronic urticaria had difficulties in attending medical care (8), the effect of state restrictions and changes in the healthcare system due to pandemic on CSU course and management are still poorly investigated.

In Russia, a broad range of restrictions (*e.g.*, social distancing, wearing a mask, *etc.*) has been applied to prevent the spread of infection. To assess the impact of these restrictions on Russian

patients with CSU, we conducted a cross-sectional online survey among adult patients diagnosed with CSU from May 5th, 2020 to June 26th, 2020 (at the end of "the first wave" of COVID-19). A 21-item survey included questions on concomitant diseases and comorbidities, time of CSU onset, severity, treatment, the impact of restrictions due to pandemic on patients' daily life, symptoms, course of the disease, treatment and access to medical care and use of telemedicine. The survey link was distributed online. The participation was voluntary and anonymous. We received 111 completed surveys meeting inclusion criteria.

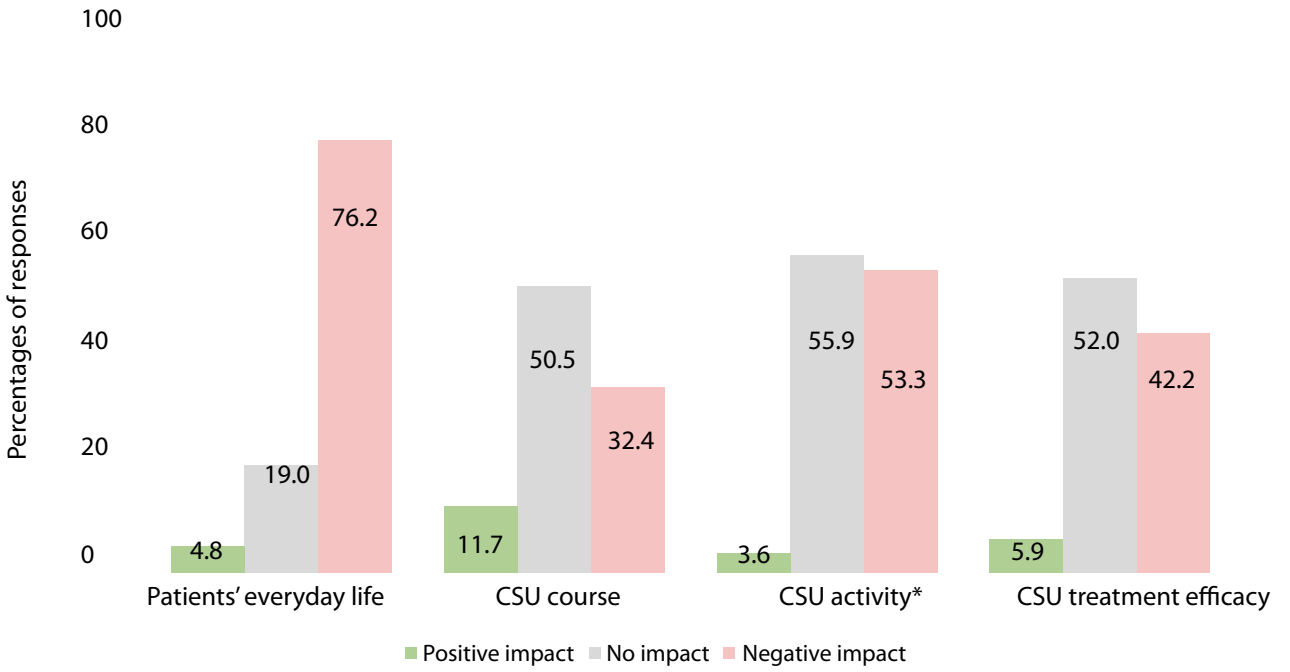
Out of 111 patients, 80.0% (89/111) were female. Median age was 33 years (interquartile range (IQR) 28-42 years) and median CSU duration was 3 years (IQR 1.6-5 years). Gastrointestinal (40.5%, 45/111), allergic (23.4%, 26/111) and cardiovascular diseases (15.3%, 17/111) were the most common reported comorbidities. Forty-four of 111 patients described that they were tested for COVID-19 and in eight of them COVID-19 was confirmed. Among COVID-19-positive patients 50.0% (n = 4) had a mild disease course and 50.0% (n = 4) had asymptomatic infection.

Negative effect of restrictions on everyday life (**figure 1**) was reported by 76.2% (80/105) patients with 15.2% (16/105) patients acknowledging severe negative and extremely negative impact. 34.2% (36/105) of respondents reported more frequent CSU exacerbations during restrictions. 54.3% of patients (56/103) associated restrictions due to pandemic with increased CSU activity. An increase in severity and frequency of pruritus, angioedema and/or wheals was noticed by 27.2% (28/103), 8.7% (9/103) and 18.4% (19/103) patients, respectively. The treatment efficacy decreased in 42.2% (43/102) of patients. One-fifth of patients (20.6%, 21/102) required an increased dose of the medication, and the frequency of medication intake increased in 13.7% (14/102) of respondents. The type of medication was changed or a new drug was introduced in 10.8% (11/102) of CSU patients. Five out of 15 patients treated with omalizumab reported low treatment efficacy due to the limited availability of omalizumab.

48.5% (52/106) patients had a limited access to in-person medical consultations. They postponed consultation (65.4%, 34/52), scheduled an appointment with another physician (7.7%, 4/52) and used telemedicine consultation as an alternative (19.2%, 10/52). Preferred options for telemedicine consultation were voice calls (50%, 6/12), messenger applications (33.3%, 4/12) and/or video calls (25.0%, 3/12). Telemedicine consultations were considered effective by 60.0% (6/10) of respondents, whereas 40% (4/10) of patients found them not helpful.

In our cohort, two-thirds of patients experienced a negative impact of pandemic on their daily life and up to a half of patients reported worsening of CSU course (**table I**, **figure 1**).

Figure 1 *- Impact of restrictions related to COVID-19 on patients' everyday life and CSU course, activity and treatment.*



*Multiple-choice questions; the diagram does not include data about the patients who failed to report.

Table I *- Factors which have the most pronounced negative impact on the course, activity and/or treatment of CSU*.*

Restrictions	% (n) of CSU patients (total n = 100)
Difficulties of getting medical aid/care	45 (45)
Stress caused by the pandemic	42 (42)
Fear of getting medical care due to self-isolation/ fear of being infected with SARS-CoV-2 or to infect others	42 (42)
Self-isolation / quarantine	25 (25)
Difficulties of buying medications	13 (13)
Restrictions of use transport / ban on the public transport use	10 (10)
No opportunity to get QR code (permission to work, visit a doctor, *etc*.), difficulties using a computer / website	3 (3)

*A multiple-choice question: patients were asked to choose the three most relevant factors related to the pandemic which impact the activity and/or severity and/or treatment of their chronic spontaneous urticaria.

The latter might be associated with restrictions directly, *e.g.,* unavailability to attend the doctor, and/or indirectly, *e.g.*, stress associated with pandemic and restrictions, that requires further investigation. In this context, telemedicine may be a valuable tool to provide the supportive care for CSU patients during the lockdown/restrictions period (9).

Fundings

None.

Contributions

NP, AA: conceptualization, investigation, methodology, resources, data curation, formal analysis, writing, review and editing. EG: data curation, formal analysis, writing. DM: conceptualization, review and editing. DS: data curation, formal analysis, writing. PK: conceptualization, investigation, methodology, review and editing.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgements

Pavel Kolkhir was supported by a GA²LEN fellowship.

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