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Urticaria and coronavirus infection: a lesson from SARS-CoV-2 pandemic

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Key words
Urticaria; allergy; SARS-CoV-2; COVID-19; MERS-CoV; SARS-CoV; viral infection.

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Summary
Urticaria is a condition involving both skin and mucosal tissues characterized by the presence of wheals and/or angioedema. The acute form has been related to allergic reactions to drugs or foods, interaction with chemicals, or infections. We reviewed the association of urticaria with coronavirus infections. This review was carried out by the use of two search engines for published original articles, employing two key terms correlated to urticaria and viruses: "urticaria" and one term linked to each virus. The research of the relationships between SARS-CoV-2 and urticaria produced 18 papers (including a total of 114 cases). Surprisingly, the search for cases of urticaria in patients with SARS-CoV or MERS produced no results. We tried to interpret this discrepancy and attempted to analyze the possible pathogenesis of urticaria lesions in SARS-CoV-2.

Introduction

Urticaria is a condition involving both skin and mucosal tissues characterized by the presence of wheals, angioedema, or both. Histologically, the wheal is characterized by edema of the external derma with a minor dilatation of the vessels, in the absence of wall injury, with a peri-vessel granulocytic infiltrate of neutrophils and eosinophils and a little number of lymphocytes and macrophages. Angioedema is defined as the quick onset of a non-inflammatory edema of the deep derma, accompanied by ache or itch, resolving within 72 h (1, 2).
Urticaria is defined as acute (AU) if it lasts less than 6 weeks while chronic urticaria (CU) lasts ≥ 6 weeks. It is estimated that 12-22% of the overall population has experienced at least one type of urticaria throughout life (3, 4). Although a precise origin is often not recognized, AU has been correlated with allergic reactions to drugs or foods, interaction with chemicals, mechanical stimulation, psychic stress, or infections. Different studies reported a prevalence ranging between 37 and 58% of infections among subjects with AU (4, 5). Upper respiratory signs and symptoms are frequent in AU associated with infections (6, 7). Viral diseases have been also associated with the onset of atopic signs and an increase of IgE levels (8). Respiratory viruses include many different families of viruses comprising *coronaviridae* (9). Recently coronaviruses have focused international attention due to the current SARS-CoV-2 pandemic. Coronaviruses were not considered to be highly pathogenic to immunocompetent humans until the epidemics of severe acute respiratory syndrome (SARS) in 2002 and 2003 in China (10) characterized by inter-human transmission of SARS-CoV and associated with elevated death rates (11).
Ten years after SARS, a different, extremely pathogenic coronavirus, the Middle East respiratory syndrome coronavirus (MERS-CoV) appeared in Middle Eastern nations (12-14). Finally, in December 2019, a novel coronavirus outbreak commenced in the city of Wuhan, China, caused by a betacoronavi-

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rus, SARS-CoV-2 (15, 16). Although it is well recognized that coronavirus disease 2019 (COVID-19) is essentially a pulmonary infection, numerous data suggest that it should be regarded as a disease involving different organs and systems, including the skin (17, 18). In the present study we reviewed the association between urticaria and coronavirus infections.

Methods

This review was carried out by using both PubMed, and Google search engines for published original and review articles. We selected articles on these Web sites, by the use of the following key terms:

a) "COVID-19", "2019-nCoV", and "SARS-CoV-2" in combination with "urticaria" or "rash" for SARS-CoV-2 infection.
b) "SARS-CoV" in combination with "urticaria" or "rash" for SARS-CoV infection.
c) "MERS" or "MERS-CoV" in combination with "urticaria" or "rash" for MERS-CoV-2 infection.

We evaluated all the studies written in English language and published in peer-reviewed journals. A main target of interest were the case reports of patients with either AU or CA correlated to coronavirus infection.We recorded the following data: author, publication year, region, number of subjects with skin manifestations, age, sex, type of infection, suspected or confirmed status for infection, cutaneous signs and their site, timeline and recovery duration, correlated symptoms, relationship between infection severity with skin lesions.

Results

After eliminating the overlaps between the two search engines, we were left with 23 works investigating the relationships between SARS-CoV-2 infection and urticaria. Of these, 18 reported cases of urticaria in 114 patients with SARS-CoV-2 infection (19-36). Unfortunately, not all studies reported the characteristics of the patients studied (gender, age, timing, *etc.*) and therefore it was not possible to perform a full analysis of the data. **Table I** summarizes some features of the patients. Among the selected papers, three larger patient series were present. Recalcati *et al.* (19) examined 88 Italian subjects: 18 (15.84 %) showed skin involvement, in 8 cases at onset before hospitalization and in 10 patients during hospital stay. Cutaneous symptoms included erythematous rashes (14 subjects), widespread urticaria (3 subjects) and chickenpox-like vesicles (1 patient). The trunk was the predominantly implicated region. Itching was weak or lacking and generally manifestations vanished in few days. There was no connection with disease severity (19).

In a Spanish study, the incidence of urticarial rash was 19% in a group of 375 SARS-CoV-2 subjects with skin manifestations and correlated to a more serious course of the infection (30). Urticaria generally developed together with other symptoms and was frequently associated with itching (92%) (30). Finally, in a large retrospective analysis of skin manifestations during SARS-CoV-2 pandemics carried out in France all 14 urticaria subjects reported had had SARS-CoV-2 infection. In these subjects, skin lesions appeared few days after the first SARS-CoV-2 systemic manifestations (33). The other works selected reported up to two cases of urticaria. Generally, the lesions vanished rapidly following the application of local corticosteroids and the use of oral antihistamines (21) or within 10 days after the onset (22).

However, urticaria must be differentiated from other conditions in which rashes or similar skin alterations can represent symptoms of underlying diseases. These include anaphylaxis, vasculitic or pigmentary urticaria, recurrent angioedema with eosinophilia, hereditary C1-inhibitor deficiency, some cutaneous expressions of ectoparasites, or granulomatous dermatitis with eosinophilia (2).

In the large analysis reported above (33), the authors reported also skin lesions other than urticaria such as chicken pox like vesicles in 2 patients, while vascular manifestations such as violaceous macules with "porcelain-like" appearance, chilblain, livedo, nonnecrotic purpura, necrotic purpura, chilblain appearance with Raynaud's phenomenon, and eruptive cherry angioma were described in 7 subjects. Forty other subjects with chilblain manifestations were described but their diagnostic PCR for SARS-CoV-2 scored negative or was not performed (33).

Surprisingly, we did not find cases of urticaria reported in patients with SARS-CoV and MERS.

Discussion

Viral infections are a potential cause of AU and COVID-19 does not represent an exception in thissense. In a recent study performed on 140 patients, urticaria was self-reported in about 1.4% of cases (37). The reason why limited data about urticaria and other respiratory viral infections are available in the literature is probably that skin lesions are short lived in most cases, which leads to a large underestimation of these phenomena. The potential severity and the worldwide spread of COVID-19, along with the availability of a precise diagnostic workout (which is missing during most other respiratory virus infections) have eventually led to accumulate an impressive amount of clinical data recording even minimal clinical signs of the disease. In most cases cutaneous lesions appear at the onset of the infection (25, 26, 31), thus skin signs may act as markers of infection in the many patients with an asymptomatic presentation of SARS-CoV-2 infection.

One reason why some patients develop urticarial skin reactions during the viral infection might be that the expression of the SARS-CoV-2 cell receptor gene angiotensin-converting enzyme 2 (ACE-2) has been reported in several human tissues including the skin (38). Moreover, viral infections may cause urticaria stimulating mast cell degranulation via complement activation (39). Furthermore, patients with AU show elevated levels of IL-6 and D-dimer, two inflammatory markers that are markedly increased during SARS-CoV-2 (40, 41). IL-6 represents the potential link between AU and infection. Urticarial rash combined with fever

Table I *- Characteristics of the patients examined.*

Authors	N. patients	Skin lesion	Timing with respect infection	Ref.
SARS-CoV-2 virus				
Recalcati *et al.*	17	Erythematous rash Urticaria	Before	19
Joob *et al.*	1	Rash	Before	20
De Medeiros *et al.*	1	Erithematous-edematous plaques	Before	21
Sachdeva *et al.*	3	Macular-papular rash Macular- papular exanthem Papular-vesicular lesions	After	22
Aktas *et al.*	1	Urticarial reaction	After	23
Fernandez-Nieto *et al.*	1	Urticarial eruption	After	24
Young *et al.*	1	Urticarial rash	Before	25
Henry *et al.*	1	Urticarial rash	Before	26
Genawan *et al.*	1	Urticaria	After	27
Rodriguez-Jimenez *et al.*	1	Urticarial eruption	After	28
Estebanez *et al.*	1	Urticarial rash	After	29
Galvan-Casas *et al.*	71	Urticarial rash	?	30
Van Damme *et al.*	2	Cutaneous rash	Before	31
Naziroglu *et al.*	1	Edematous plaques	Before	32
Bouaziz *et al.*	7	Exanthema Cold urticaria Chickenpox like vesicles	After	33
Adelino *et al.*	1	Wheels Facial angioedema	After	34
Najafzadeh *et al.*	1	Urticaria and angioedema	Before	35
Guarneri *et al.*	2	Urticaria	?	36

might be suggestive of SARS-CoV-2 infection (42). The possibility that drugs administered to SARS-CoV-2-infected patients may be involved in the appearance of urticaria should be also carefully considered. A severe drug-induced skin reaction similar to an acute systemic exanthematous pustulosis subsequent to hydroxychloroquine treatment has been reported. It can be distinguished by its longer incubation time, and more heterogenous morphology firstly frankly urticarial in nature and subsequently characterized by targetoid and arcuate plaques, and by its resistance to treatment (22). Moreover, most patients with COVID-19 take acetaminophen, nonsteroidal antinflammatory drugs and antibiotics during the first phases of the disease. It is certainly conceivable that the administration of these drugs may also play a role in the onset of urticarial manifestations, at least in some patients.

Conclusions

In conclusion, clinicians must be aware that urticaria may present during COVID-19 possibly in patients with a severe clinical course of the disease. The identification of this condition might lead to an improvement in the diagnosis and therapy of COVID-19 as well as in a more rapid application of quarantine practices.

Conflict of interests

The authors declare that they have no conflict of interests.

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Correlations between disease activity, autoimmunity and biological parameters in patients with chronic spontaneous urticaria

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Key words

Chronic spontaneous urticaria; blood basophil count; IgE; autoimmunity; autologous serum skin test.

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Abbreviations
AAbs: autoantibodies
ANA: anti-nuclear antibodies
ASST: autologous serum skin test
BAT: basophil activation test
BHRA: basophil histamine release assays
CBC: complete blood count
CRP: C-reactive protein
CSU: chronic spontaneous urticaria
FceRI: high-affinity IgE receptor
IgE: immunoglobuline E
RF: rheumatoid factor
Tg: thyroglobuline
TPO: thyroperoxydase
UAS7: Weekly Urticaria Activity Score

Summary

Background. *Biomarkers of disease activity/severity and criteria of autoimmune chronic spontaneous urticaria (CSU) are still a matter of debate.* ***Objective.*** *To investigate possible correlations between clinical and biological markers and their associations with: 1) disease activity, 2) resistance to H_1-antihistamines, 3) autoimmunity and 4) autologous serum skin test (ASST) in patients with CSU. To also analyze biological parameter modifications in patients with CSU treated with omalizumab.* ***Materials and methods.*** *Disease activity, H_1-antihistamines response and presence of concomitant autoimmune disease were prospectively recorded in 95 patients with CSU. For 60 of them, ASST was performed. Broad biological analysis were performed.* ***Results.*** *C-reactive protein (CRP) serum levels were higher in H_1-antihistamines unresponders ($p < 0.0001$) and in more active diseases ($p = 0.033$). D-dimer plasma levels were higher in H_1-antihistamines unresponders ($p = 0.008$) and in patients with autoimmune status (concomitant autoimmune disease and/or with autoantibodies) ($p = 0.016$). Total immunoglobuline E (IgE) serum level was lower in patients with positive ASST. Blood basophil counts were lower in patients with CSU and especially in H_1-antihistamines unresponders ($p = 0.023$), in patients with more active disease ($p = 0.023$), with positive ASST ($p = 0.001$), and with autoimmune status ($p = 0.057$). Conversely, under omalizumab, a decrease of CRP ($p = 0.0038$) and D-dimer serum/plasma levels ($p = 0.0002$) and an increase of blood basophil counts ($p = 0.0023$) and total IgE serum levels ($p = 0.0007$) were observed.* ***Conclusions.*** *This study brings additional evidences of interest to investigate IgE, D-dimer serum/plasma levels and basophil blood counts in patients with CSU as they could be correlated to disease activity, response to treatment and/or autoimmunity.*

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Introduction

Chronic spontaneous urticaria (CSU) is defined as the spontaneous occurrence of wheals and/or angioedema daily or almost daily for more than 6 weeks. The pathogenesis of CSU has not been fully established although it seems clear that different mechanisms are involved. Mast cells have long been the key cells involved in CSU pathogenesis, however new evidence argues in favor of the involvement of other cells, *i.e.,* basophils, eosinophils, lymphocytes, and neutrophils, as well as the involvement of cytokines, coagulation pathways and autoantibodies (AAbs). Autoimmune diseases, particularly autoimmune thyroiditis and thyroid AAbs, seem more prevalent in patients with CSU (1, 2). Several AAbs have been associated with CSU: IgG against thyroperoxydase (TPO) or thyroglobuline (Tg) (1, 3), IgG against IgE or against high-affinity IgE receptor (FceRI) (4, 5), and IgE directed against autoantigens, such as TPO or interleukin 24 (6, 7). Furthermore, some patients with CSU react to the intradermal injection of their own serum resulting in a positive autologous serum skin test (ASST) (8). Also *in vitro* tests (basophil histamine release assays (BHRA) and basophil activation test (BAT)) showed that some CSU serum factors are able to induce histamine release/basophil activation (5, 9). The above-mentioned factors, alone or combined, are often used to classify patients as autoimmune or non-autoimmune CSU.

In this prospective cohort of patients with CSU, we analyzed correlations between several clinical and biological markers and their associations with: 1) disease activity, 2) response to H_1-antihistamines, 3) autoimmunity, 4) ASST. We also analyzed biological parameter modifications in patients with CSU treated with omalizumab (anti-IgE treatment).

Materials and methods

This prospective study was conducted from September 2013 to December 2018 in the department of Dermatology of the Cliniques universitaires Saint-Luc, in Brussels, Belgium. The study and data collection were conducted with the approval of the institutional ethical committee. Informed consent was obtained from all patients.

Patient selection and clinical data collection

Ninety-five adults and adolescents (≥ 12 years old) with a diagnosis of CSU, confirmed by a dermatologist according to the international Guideline 2013 (10), were included. Only patients with active CSU were selected. Patients with pure chronic inducible urticaria or bradykinin-mediated angioedema were not included in the study. Upon enrolment, medical history, including history of personal or familial atopy (asthma, atopic dermatitis and allergic rhinitis) and autoimmune diseases, as well as previous and current treatments for CSU were recorded. Using a validated tool, the Weekly Urticaria Activity Score (UAS7), assessed disease activity (10). Patients were asked to record their symptoms for seven consecutive days prior to day of inclusion. Patients were classified as follows: severe CSU (UAS7 = 28-42), moderate CSU (UAS7 = 16-27), mild CSU (UAS7 = 7-15), well-controlled CSU (UAS7 = 1-6) and itch-and wheals-free (UAS7 = 0) (11).

Response to H_1-antihistamines was also evaluated and assessed using the UAS7 over several months. Patients were classified as follows: H_1-antihistamines responders (UAS7 ≤ 7 with 1 to 4 tablets daily of H_1-antihistamines); H_1-antihistamines unresponders (UAS7 > 7 with 4 tablets daily of H_1-antihistamines).

Disease duration was defined as the time from the first onset of symptoms to day of inclusion. Recurring episodes of CSU, defined as recurrence of symptoms after at least 6 months of spontaneous remission, were also recorded.

For ASST and blood analyses, patients were considered as untreated, if they had stopped H_1-antihistamines for at least 48 hours (or longer, depending on drug activity of each molecule) (8), anti-leukotrienes and H_2-antihistamines for at least 7 days, and corticosteroids or cyclosporine A for at least 1 month and have never taken omalizumab before inclusion.

Autoimmunity and autologous serum skin test

Patients were also classified according to their "autoimmune status". Autoimmune status was inferred in the case of a personal history of concomitant autoimmune disease or in the presence of at least one type of AAbs (included IgG against Tg and TPO, anti-nuclear antibodies (ANA) and rheumatoid factor (RF)). No autoimmunity was defined as the absence of concomitant autoimmune disease and AAbs.

ASST was performed on 60 untreated patients by the intradermal injection of 50 µL of the patient's own serum into the volar part of the forearm (8). Prick tests with histamine and intradermal injection of normal saline solution served as respectively positive and negative controls. A positive test was defined as the appearance, within 30 minutes, of a red wheal with a diameter of 1.5 mm or greater than the wheal produced by the injection of normal saline solution. Patients were classified as either having a positive or a negative ASST.

Biological tests

Blood analyses include: complete blood count (CBC) with differential, platelet parameters, total IgE serum levels, thyroid function tests, serum levels of IgG against Tg and TPO, ANA, RF, C-reactive protein (CRP) serum levels, complement components (C3, C4), C1-inhibitor, classical complement pathways, protein electrophoresis, and D-dimer plasma levels. Titers were considered positive if IgG anti-Tg > 115 U/ml, IgG anti-TPO > 34 U/ml, ANA > 1:160, and RF > 1:40. For basophil blood counts, the reference range often used is from 0 to 200 or 300/

µL. As this range is very large and start from zero, we used a reference mean for blood basophil counts which was established by the department of Clinical Biology of the Cliniques universitaires Saint-Luc based on healthy controls values.

Omalizumab treatment

For 22 patients treated with omalizumab (Xolair , Novartis, Camberley, UK) at the initial recommended dose of 300 mg every 4 to 5 weeks (12), blood tests were performed before initiation and under omalizumab treatment.

Patients were classified according to their response to omalizumab treatment as follows: complete responders if UAS7 was 0, partial responders if UAS7 fell by at least 10 points (but UAS7 0), and non-responders if UAS7 remained unchanged, rose, or fell by less than 10 points. Based on time to response, patients were classified as early responders, if their UAS7 fell by at least 10 points after one month of treatment. Others were classified as late responders.

Statistical analyses

As some clinical or biological data may be missing for some patients, the number of patients studied for each parameter is always indicated. Data for categorical variables are expressed as frequencies followed in brackets by the number patients positive for this parameter over number of patients studied, and for continuous variables as mean ± standard deviation (SD) with minimum and maximum values in brackets. The Pearson's x^2 test was applied to compare percentages of categorical variables. Mann-Whitney test and Kruskal-Wallis test were used to compare continuous variables between categorical variables. Wilcoxon matched-pairs signed rank test was used to compare paired variables, such as values before initiation and under omalizumab. The Pearson correlation coefficient was used to calculate correlation between continuous variables. In all tests, the level of significance was a two-sided P value of less than 0.05. All statistical analyses were performed, and graphs created using SPSS software Version 24 (SPSS, Chicago, IL, USA) and GraphPad Prism Version 8 (GraphPad Software Inc., USA).

Results

Relevant patient data **(table I)**

Clinical data

This study included 95 patients with CSU, 68 women (71.6%) and 27 men (28.4%). Mean age at inclusion was 45 ± 16 years and mean duration of CSU was 4.7 ± 7 years. Mean age of CSU onset was 40 ± 16 years. Angioedema was associated with wheals in 66 patients (69.5%). Recurring episodes of CSU after at least 6 months of symptom free intervals without treatment were reported in 25.3% of patients (23/91). 58.9% (56/95) were H_1-antihistamines responders and 41.1% (39/95) were H_1-antihistamines unresponders. Concerning disease activity based on UAS7, 24 patients (40.7%) have severe disease, 10 patients (16.9%) have moderate disease, 17 patients (28.8%) have mild disease, 3 patients (5.1%) have well-controlled disease and 5 patients (8.5%) were itch-and wheals-free. ASST was performed on 60 patients and 24 (40%) were positive.

Biological parameters

Biological tests that could be influenced by treatment (*e.g.*, cell blood counts, platelet parameters, D-dimer plasma levels, IgE and CRP serum levels, complement components) were analysed only in untreated patients. Mean serum IgE levels was 208.2 ± 451.8 kU/L, with 43.7% (31/71) of patients having levels higher than 150 kU/L and 23.9% (17/71) having levels lower than 40 kU/L. Mean D-dimer plasma levels was 1278.2 ± 1939 ng/ml, with 56.3% (40/71) of patients having levels higher than 500 ng/ml. For both IgE and D-dimer levels, the mean values observed were higher than normal ranges and large variability was seen across patients. Blood basophil counts were lower in patients with CSU (30.1 ± 24/µL) (74 patients) compared with reference mean of healthy controls (40.0 ± 17.3/µL) ($p = 0.019$). No significant abnormalities were found in the rest of the CBC, in protein electrophoresis, nor in complement.

Associations with autoimmune disease, autoimmune serology or atopy

One third of the patients (32/94, 34.0%) had clinical history and/or serological markers of autoimmunity, and therefore were considered as having a positive autoimmune status. Indeed, a concomitant autoimmune disease was present in 18.3% (17/93) of patients, mainly thyroiditis (11/17), and AAbs were present in 30.5% (29/95), predominately anti-TPO (20/92). As well, familial history of autoimmune disease was found in 14,6% of cases (13/89). Nearly half of the patients (45/91, 49.5%) had a personal history of atopy (based on anamnesis).

Correlations between disease activity, response to H_1-antihistamines and clinical or biological parameters

Response to H_1-antihistamines was not correlated with clinical parameters such as angioedema, symptomatic dermographism, duration of the disease, age, weight, gender, personal or family history of atopy. In addition, no association was found between H_1-antihistamines response and concomitant autoimmune disease, presence of AAbs, the positivity of ASST nor blood cell counts or total IgE serum levels.

Table I *- Clinical and biological data of the cohort of patients with CSU.*

	N studied	Numbers (%) or mean ± SD (min-max)	Reference value
Sex female	95	68 (71.6%)	
Age at inclusion (years)	95	45.1 ± 16.2 (13.7-91.5)	
Age of onset (years)	95	40.4 ± 16.4 (10.1-86.6)	
Disease duration		4.7 ± 7 years (2 months-38 years)	
Period of remission ≥ 6 months	91	23 (25.3%)	
Angioedema	95	66 (69.5%)	
Symptomatic dermographism	23	15 (65.2%)	
Personal history of atopy	91	45 (49.5%)	
Familial history of atopy	89	39 (43.8%)	
Personal history of concomitant autoimmune disease	93	17 (18.3%)	
Thyroiditis	17	11 (64.7%)	
Vitiligo	17	3 (17.6%)	
Thyroiditis + Vitiligo	17	1 (5.9%)	
Alopecia areata	17	1 (5.9%)	
Idiopathic thrombopenia purpura	17	1 (5.9%)	
Familial history of autoimmune disease	89	13 (14.6%)	
Positivity of ASST	60	24 (40%)	
CRP serum levels (mg/L)	71	7.6 ± 13.3 (1-78)	< 5
D-dimer plasma levels (ng/ml)	71	1278.2 ± 1939 (250-12687)	< 500
Blood cells counts			
leukocytes (x 10^3/µL)	74	7.52 ± 2.94 (3.26-24.12)	(4.0-10.0)
neutrophils (x 10^3/µL)	74	4.77 ± 2.48 (1.31-18.17)	(1.6-7)
lymphocytes (x 10^3/µL)	74	1.92 ± 0.55 (0.74-3.67)	(0.8-5)
monocytes (x 10^3/µL)	74	0.48 ± 0.14 (0.16-0.84)	(0.2-1)
eosinophils (x 10^3/µL)	74	0.14 ± 0.09 (0-0.47)	(80-600)
basophils (x 10^3/µL)	74	0.03 ± 0.02 (0-0.15)	(0-0.2)
platelets (x 10^3/µL)	74	265.43 ± 76.69 (125-694)	(150-350)
Platelet parameters			
mean platelet volume (µm^3)	74	10.6 ± 1 (8.9-13.4)	(9.1-11.9)
PDW (fL)	74	12.5 ± 2.2 (9.4-19.1)	(9.9-15.4)
plateletcrit (%)	74	0.3 ± 0.1 (0.1-0.7)	(0.17-0.35)
ratio large platelet (%)	74	29.5 ± 8.1 (16.5-53.1)	(17.5-42.3)
Total IgE serum levels (kU/L)	71	208 ± 451.8 (2-3656)	< 150
Positivity of AAbs	95	29 (30.5%)	
AAbs anti-Tg	92	15 (16.3%)	
AAbs anti-TPO	92	20 (21.7%)	
ANA	90	9 (10%)	

Table I *- Clinical and biological data of the cohort of patients with CSU.*

		N studied	Numbers (%) or mean ± SD (min-max)	Reference value
Rheumatoid factor		91	2 (2.2%)	
Autoimmune status		94	32 (34.0%)	
H_1-antihistamines	responders	95	56 (58.9%)	
	unresponders	95	39 (41.1%)	
UAS7		59	21.0 ± 12.6 (0-42)	
Activity based on UAS7				
28-42: severe		59	24 (40.7%)	
16-27: moderate		59	10 (16.9%)	
7-15: mild		59	17 (28.8%)	
1-6: well-controlled		59	3 (5.1%)	
0: itch-and wheals-free		59	5 (8.5%)	

Autoimmune status: concomitant autoimmune disease and/or AAbs.
H_1-antihistamines response: H_1-antihistamines responders (UAS7 7 with 1 to 4 tablets daily of H_1-antihistamines); H_1-antihistamines unresponders (UAS7 > 7 with 4 tablets daily of H_1-antihistamines).
Weekly Urticaria Activity Score (UAS7) was recorded by patient for seven consecutive days prior to sampling day. Patients were classified according UAS7 as follows: severe CSU (UAS7 = 28-42), moderate CSU (UAS7 = 16-27), mild CSU (UAS7 = 7-15), well-controlled CSU (UAS7 = 1-6) and itch-and wheals-free (UAS7 = 0).
Blood analyses that can be influenced by treatment (blood cells, platelet parameters, D-dimer plasma level, IgE and CRP serum levels, complement components) were recorded for untreated patient.
Titers for AAbs were considered positive if anti-Tg >115 U/ml, anti-TPO > 34 U/ml, ANA > 1:160, and RF > 1:40. Cut-off for CRP serum level, for D-dimer plasma level and for total IgE serum level detection were respectively 1 mg/L, 250 ng/mL and 2 kU/L.

Conversely, in H_1-antihistamines unresponders, CRP serum levels ($p < 0.0001$) (**figure 1 A**) and D-dimer plasma levels ($p = 0.009$) (**figure 1 B**) were significantly higher than in H_1-antihistamines responders. Moreover, CRP serum levels and D-dimer plasma levels were positively correlated ($p < 0.0001$).
Disease activity (based on UAS7) was positively correlated to CRP serum levels ($p = 0.033$) (**figure 1 C**) and negatively correlated to blood basophil counts ($p = 0.023$) (**figure 1 D**). Correlation between D-dimer plasma levels and UAS7 did not reach significance ($p = 0.069$).
Disease activity was not associated with clinical parameters nor with the rest of CBC values, total IgE serum level nor with platelet parameters.

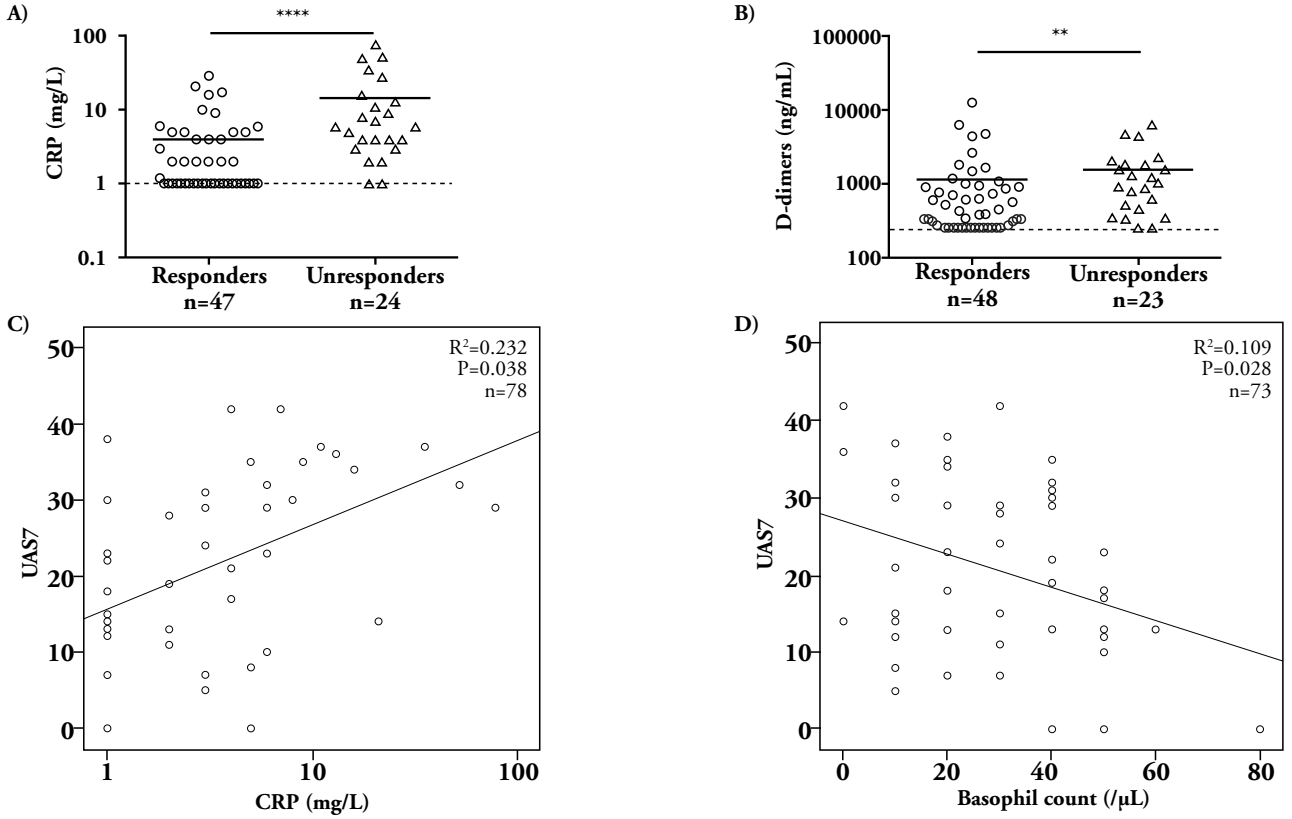
Correlation between autoimmune or autoreactive factors and biological parameters

An association between positive ASST results and autoimmune status (defined as the presence of concomitant autoimmune disease and/or AAbs) was observed ($p = 0.037$).
Positivity of ASST was correlated with angioedema ($p = 0.005$) as 87.5% (21/24) of patients with positive ASST had angioedema in contrast to 52.8% (19/36) with negative ASST.
Blood basophil counts (**figure 2 A**), blood monocyte counts, and mean total IgE serum levels (**figure 2 B**) were lower in patients with positive ASST (respectively $p = 0.001$, $p = 0.019$ and $p = 0.016$). However, other clinical and biological parameters were not correlated with ASST results.
D-dimer plasma levels were higher ($p = 0.016$) (**figure 2 C**), and blood basophil counts lower ($p = 0.057$) (**figure 2 D**) when an autoimmune status was present. Blood platelet counts and plateletcrit were also higher in patients with autoimmune status (respectively $p = 0.010$ and $p = 0.002$). Other clinical and biological parameters were not correlated with autoimmune status.

Omalizumab: before initiation and under treatment

For 22 patients, biological parameters were compared before initiation and under omalizumab. 11 patients were complete responders, 8 partial responders and 3 non-responders to omalizumab. Among responders, 17 patients were early responders

Figure 1 - (A) *Positive correlation between CRP serum levels and H_1-antihistamines response. Responders: 4.0-5.7 mg/L (1-29); unresponders: 14.7-19.9 mg/L (1-78).* ***(B)*** *Positive correlation between D-dimer plasma levels and H_1-antihistamines response. Responders: 1144-2093 ng/mL (250-12687); unresponders: 1558.5-1574.3 ng/mL (250-6260).* ***(C)*** *Positive correlation between CRP serum levels and disease activity (UAS7).* ***(D)*** *Negative correlation between blood basophil counts and disease activity (UAS7). Untreated patients. UAS7 recorded seven consecutive days before the blood sample.*



Each symbol represents one patient. Solid horizontal line represents mean.
P-value: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, blank p > 0.05.
Cut-off for CRP serum level and for D-dimer plasma level detection were respectively 1 mg/L and 250 ng/mL (dotted lines).

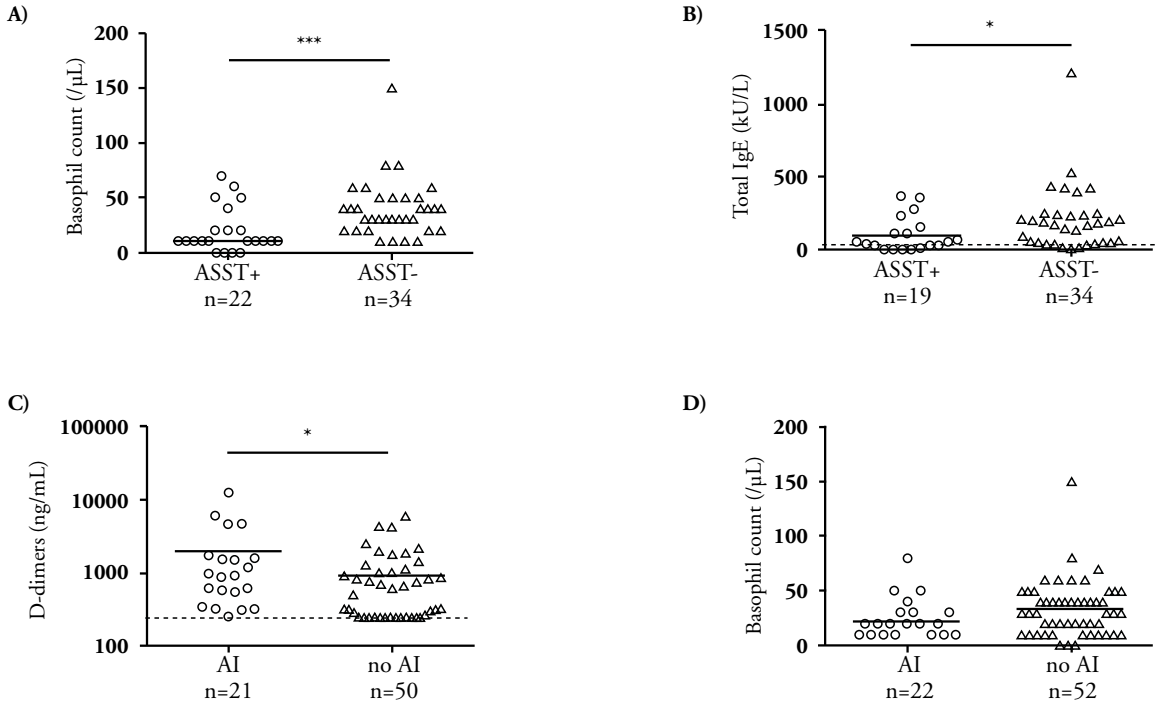
and 2 were late ones. All data concerning patients before initiation and under omalizumab treatment are reported in **table II**. Significant reductions in CRP serum levels (p = 0.0038) and D-dimer plasma levels (p = 0.0002) were observed under omalizumab treatment, whereas increases were observed in blood basophil counts (p = 0.0023) (**figure 3**) and total IgE serum levels (p = 0.0007). Blood basophil counts increased after omalizumab in 13/19 patients, with a mean increase of 113% (20-200%). No differences for the rest of CBC and platelet parameters were observed.

Neither clinical, nor biological parameters (including ratio of these parameters under omalizumab and before initiation) were associated with omalizumab response or with delay of response. However, the number of patients in each group was insufficient to have a statistically significant analysis.

Discussion

Since nearly 30 years, several lines of evidence argue for an autoimmune basis of CSU, or at least in a subgroup of them. However, the way to distinguish autoimmune and non-autoimmune CSU is still a matter of debate (13, 14). In this study, we focused on correlations between several biological parameters, concomitant autoimmune disease and/or presence of AAbs (included IgG anti-Tg, IgG-TPO, ANA and RF) and positivity of ASST. We found a relatively high incidence of autoimmune disorders and AAbs (autoimmune status) in patients with CSU. One

Figure 2 - (A) *Lower blood basophil counts in patients with ASST + than with ASST -. ASST +: 19.6-20.6/µL (0-70); ASST-: 39.7-26.6/µL (10-150).* ***(B)*** *Lower total serum IgE levels in patients with ASST + than ASST -. ASST +: 103.4-120.2 kU/L (2-368); ASST -: 207.3-225.5 kU/L (10-1216).* ***(C)*** *Higher D-dimer plasma levels in patients with autoimmunity than patients without autoimmunity. AI: 2050-2952 ng/ml (256-12687); nonAI: 953. 9-1205 ng/ml (250-6207).* ***(D)*** *Lower blood basophil counts in patients with autoimmunity than patients without autoimmunity. AI: 22.7-18.8/µL (0-80); nonAI: 33.3-25.4/µL (0-150). ASST + and ASST -: patients with positive or negative ASST respectively. AI and noAI: patients with autoimmune status (concomitant autoimmune disease and/or positive for at least one AAbs) and patients without autoimmune status (no concomitant autoimmune disease, no AAbs).*



Each symbol represents one patient. Solid horizontal line represents mean.
P-value: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, blank p > 0.05.
Cut-off for total IgE serum level and D-dimer plasma level detection were respectively 2kU/L and 250ng/mL (dotted lines).

third of the patients had concomitant autoimmune disease and/or AAbs, mainly autoimmune thyroiditis and IgG against TPO. Moreover, a familial history of autoimmune disease was also found in 14,6% of patients. Recently, Schoepke *et al.* showed that autoimmune CSU (defined by the presence of IgG anti-IgE or anti-FceRI, a positive BAT and a positive ASST) have significantly higher IgG against TPO than patients with non-autoimmune CSU (15). In our cohort, we found a correlation between positive ASST and presence of concomitant autoimmune disease and/or AAbs. This association was not always found in previous studies, a discrepancy which could be explained by the fact that we got interest for both; presence of concomitant autoimmune disease and AAbs (16-19). The proportion of positive ASST in our cohort of patients with CSU (40%) is consistent with previous reports (30% to 50%) (8, 20). Presence of concomitant autoimmune disease and/or AAbs or positivity of ASST was not correlated with disease activity nor with H_1-antihistamines response. In the literature, this association between ASST and disease activity remains controversial (16, 20-22). In line with previous reports, angioedema was more frequent in patients with positive ASST in our cohort (17, 18). This study put forward that personal and familial autoimmune disease history as well as autoimmune serology, especially IgG against TPO, are easy to get and could be interesting to record in patients with CSU.

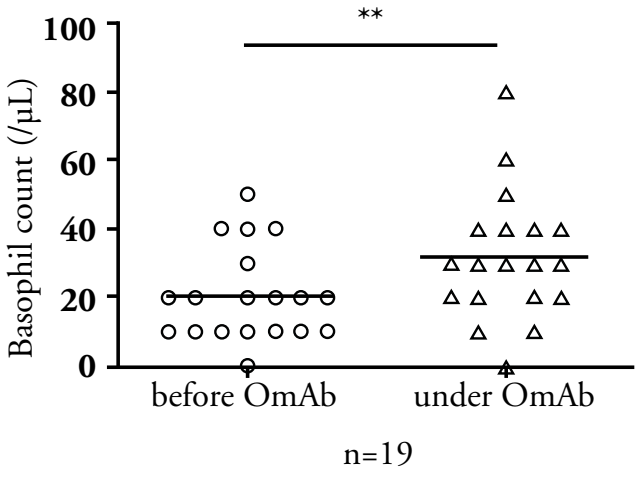
As several studies had postulated that CRP and IgE serum levels or D-dimer plasma level could be considered as biomarkers of CSU or CSU activity, we have measured them and looked for

Table II *- Clinical and biological data for patients treated with omalizumab. Comparison before initiation and under omalizumab.*

	All omalizumab patients				
	N studied	**Number (%) or mean ± SD (min-max)**			
Sex female	22	17 (77.3%)			
Age (years)	22	42.7±14.2 (13.7-70.2)			
Weight (kilogrammes)	4	77.3 ± 23.8 (61-112)			
Angioedema	22	16 (72.7%)			
Duration of disease (years)	22	4.5 ± 7.9 (0.5-38.3)			
Period of remission ≥ 6 months	22	2 (9.1%)			
Autoimmune status	22	9 (40.9%)			
Positivity of ASST	10	4 (40%)			
	Before omalizumab initiation		**Under omalizumab**		
	N studied	**number (%) or mean ± SD (min-max)**	**N studied**	**number (%) or mean ± SD (min-max)**	**P-value**
UAS7	18	31.4 ± 7.4 (17-42)	12	6.3 ± 11.4 (0-38)	
Activity based on UAS7					
28-42: severe	18	14	12	1	
16-27: moderate	18	4	12	1	
7-15: mild	18	0	12	2	
1-6: well-controlled	18	0	12	2	
0: itch-and wheals-free	18	0	12	6	
CRP serum levels (mg/L)	18	18 ± 21.9 (1-78)	18	5.1 ± 5.4 (1-23)	0.0038
D-dimer plasma levels (ng/ml)	17	1668 ± 1795 (250-6260)	17	397.1 ± 307.8 (250-1248)	0.0002
Leukocytes (x 10^3/µL)	19	7.9 ± 2.4 (4.7-12.2)	19	7.4 ± 2.1 (4.3-12)	0.35
Neutrophils (x 10^3/µL)	19	5.2 ± 2.1 (2.4-9.1)	19	4.6 ± 1.6 (2.4-8.2)	0.10
Lymphocytes (x 10^3/µL)	19	2.1 ± 0.7 (0.7-3.7)	19	2.1 ± 0.7 (0.6-3.8)	
Monocytes (x 10^3/µL)	19	0.5 ± 0.2 (0.2-0.7)	19	0.5 ± 1.1 (0.3-0.7)	0.25
Eosinophils (x 10^3/µL)	19	0.1 ± 0.08 (0-0.3)	19	0.1 ± 0.06 (0-0.2)	0.83
Basophils (x 10^3/µL)	19	0.02 ± 0.01 (0-0.05)	19	0.03 ± 0.02 (0-0.08)	0.0023
Platelets (x 10^3/µL)	19	282 ± 58.2 (196-378)	19	275.7 ± 39.3 (216-359)	0.75
Mean platelet volume (µm^3)	19	10.5 ± 0.9 (9.1-12.6)	19	10.7 ± 0.8 (9.1-12)	0.15
Total IgE serum levels (kU/L)	18	137.4 ± 121.9 (2-425)	18	458.6 ± 420.7 (2-1243)	0.0007
Response to omalizumab					
complete responders			22	11 (50%)	
partial responders			22	8 (36.4%)	
non responders			22	3 (13.6%)	
Time to respond to omalizumab					
early responders			19	17 (89.5%)	
late responders			19	2 (10.5%)	

Autoimmune status: concomitant autoimmune disease and/or AAbs.
Weekly Urticaria Activity Score (UAS7) was recorded by patient for seven consecutive days prior to sampling day.
Titers for AAbs were considered positive if anti-Tg >115 U/ml, anti-TPO > 34 U/ml, ANA > 1:160, and RF > 1:40. Cut-off for CRP serum level, for D-dimer plasma level and for total IgE serum level detection were respectively 1 mg/L, 250 ng/mL and 2 kU/L.

Figure 3 *- Elevation of blood basophil counts under omalizumab treatment. Before omalizumab initiation: 20.5 ± 13.5/µL (0-50); under omalizumab: 31.6 ± 18.6/µL (0-80).*



Each symbol represents one patient. Solid horizontal line represents mean. P-value: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, blank p > 0.05. Before OmAb: patients before beginning of omalizumab treatment; under OmAb: patients under omalizumab.

correlations with each other, and with clinical data in this prospective study of 95 CSU patients.

CRP serum level has been proposed as a biomarker of disease activity in patients with CSU, however results are contradictory (23, 24.) Our findings show that CRP (mean CRP 7.7 mg/L) was higher in CSU patients with more active disease and in H_1-antihistamines unresponders. However, to our opinion, CRP is not an interesting biomarker in CSU due to its relatively low levels compared with others inflammatory diseases and due to possible fluctuations by intercurrent phenomena which are not always reported especially in retrospective studies. CRP is more useful in the differential diagnoses of CSU, a high CRP level, not reducing under treatment, could be a sign of an auto-inflammatory syndrome.

Several studies have shown 1) higher D-dimer plasma levels in patients with CSU compared with HCs, 2) higher D-dimer plasma levels in more active disease, as well as 3) normalization of D-dimer plasma levels during remission (25, 26). In our cohort, D-dimer plasma levels were higher in H_1-antihistamines unresponders and to a lesser extent, in more active disease. Interestingly, we also found that patients with autoimmune status had higher D-dimer plasma levels than those without autoimmunity. Furthermore, in line with previous reports, we found a significant decrease of D-dimer plasma levels under omalizumab treatment. Baseline D-dimer plasma levels do not predict the response to omalizumab (27-29), whereas the decrease of D-dimer plasma levels under omalizumab treatment seem to be a marker of good response to treatment (30). Measurement of D-dimer plasma levels could be added to clinical tools, such as UAS7, to evaluate activity/severity. Concerning response to treatment, as well as relation between D-dimer plasma levels and autoimmunity, further studies are needed.

Mean total IgE serum levels have often been studied in patients with CSU and have been proposed as a biomarker of disease activity, however, results remain contradictory (31, 32). In our study, the mean total IgE serum levels of untreated patients were in normal range or little high (208.2 ± 451.8 kU/L, except in atopic patients for which is high), and less than half of patients had total IgE serum levels higher than normal reference value. Total IgE serum levels didn't correlate with disease activity nor with response to H_1-antihistamines. Interestingly total IgE serum levels were lower in patients with positive ASST (mean 103.37 ± 120.25 kU/L) compared with negative ASST (mean 207.32 ± 225.51 kU/L) ($p = 0.016$). This finding is in line with recent publications showing that very low total IgE and positive ASST have been relat ed with poor/no response and with slow response to omalizumab respectively (27, 33-36). Moreover, Schoepke *et al.* put forward that autoimmune CSU have significantly lower total IgE serum levels than non-autoimmune CSU (15). Low baseline IgE has been described as a marker of poor response (27, 34, 37). However this has not been confirmed by all studies (38, 39). Interestingly, a recent paper suggest that total IgE levels can be used as predictors of response to omalizumab only in nonatopic CSU patients, actually they showed that the atopic status modify the ability of IgE to predict the response to the treatment (40).

Several authors have discussed a possible main role of basophils. Indeed, in our study, we found that blood basophil counts were significantly lower in patients with CSU compared with healthy controls. Moreover, blood basophil counts correlated with disease activity. Basophils are probably recruited into the skin during wheal formation, as evidenced by an abundance of basophils in skin samples (41, 42), and low blood basophil counts in patients with chronic urticaria (43, 44). Furthermore, in our study, blood basophil counts were significantly lower in patients with positive ASST, and also tended to be correlated with presence of concomitant autoimmune disease and/or AAbs. This correlation has previously been poorly investigated and with controversial results (21, 43, 45, 46). Nevertheless, expression of activation markers, such as CD203c and CD63, has been found to be higher in blood basophils of CSU patients with positive ASST compared to patients with negative ASST (45, 47). It is tempting to speculate that in patients with positive ASST, basophils are implicated and activated in a more important way, and thus reduced in blood due to recruitment into the skin. Mechanisms implicated in basophils activation/recruitment in skin are actually unknown, however according to previous findings, AAbs could be indirectly implicated.

Additionally, under omalizumab treatment, we observed a significant increase in blood basophil counts, suggesting that omalizumab blocks this basophil shift from the bloodstream into the skin. Unfortunately, our cohort of patients was too small to identify differences between responders/unresponders and fast/slow responders to omalizumab. In the same line, previous authors have already observed increased blood basophil counts in correlation with improvement on treatment or remission (48, 49). However, data concerning blood basophil counts in patients with CSU under omalizumab treatment are scarce (50-53).
Consistence of this study is to have analyzed several biomarkers, evaluated in untreated patients, as well as a series of clinical parameters in a prospective cohort. Limitations are mainly due to the fact that correlations between clinical/biological parameters and omalizumab response (or delay of response) were not possible due to the small number of patients in each group. Secondly, concerning autoimmune CSU investigations, we haven't performed functional tests as BAT and BHRA nor IgG anti-FcεRI/IgE measurement.

Conclusions

To conclude, in this prospective study, we found a relatively high incidence of concomitant autoimmune disease and AAbs. D-dimer plasma level is higher in H_1-antihistamines unresponders and in patients with autoimmune status. Total IgE serum levels were lower in patients with positive ASST compared with negative ASST. We found lower blood basophil counts in patients with CSU compared with healthy controls. Moreover, this finding was more significant in patients with positive ASST and to a lesser extent in patients with autoimmune status. Moreover, under omalizumab, blood basophil counts and total IgE serum levels increased and conversely D-dimer plasma levels decrease. Our study brings additional evidences over the utility of those clinical and biological parameters to investigate in patients with CSU as they could be related to disease activity, response to treatment or autoimmunity.

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Conflict of interests

L.d.M. reports medical investigator/advisor and educational activities for Novartis. A-S.D. reports educational activities for Novartis. A.G.A. acted as medical advisor for Uriach Pharma, Genentech, Novartis, FAES, GSK, Sanofi. A.G.A reports research Grants supported by Uriach Pharma, Novartis, Grants from Instituto Carlos III- FEDER and educational activities for Uriach Pharma, Novartis, Genentech, Menarini, LEO- PHARMA, GSK, MSD, Almirall, and Sanofi. M.B. reports medical investigator/advisor and educational activities for Novartis.

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Clinical trial to assess tolerability and subrogate efficacy effects of an abbreviated schedule with house dust mites mixture subcutaneous immunotherapy

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Key words

House dust mites; DPT/DF mixture; subcutaneous immunotherapy; rhinoconjunctivitis; abbreviated schedule.

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Summary

Objective. *To evaluate the tolerability and efficacy of Dermatophagoides pteronyssinus/Dermatophagoides farinae mixture subcutaneous immunotherapy (SCIT).* ***Methods.*** *Patients received an abbreviated build-up schedule. The aims were: number, percentage, and severity of adverse reactions. Secondary outcomes included: changes in immunoglobulin titers and changes in dose-response skin prick tests.* ***Results.*** *Out of 289 administrations, 17% elicited any clinically relevant adverse reaction. Most of them were local reactions (LR) (9.4%) and the rest (7.6%) were systemic. Significant increases in sIgG and sIgG4 were detected in serum samples. Cutaneous reactivity decreased significantly.* ***Conclusions.*** *SCIT with house dust mites mixture of ROXALL Medicina España S.A. seems to have an acceptable tolerability profile, induces blocking IgG and decreases skin reactivity.*

Abbreviations

AEs: Adverse events
AIT: Allergen immunotherapy
AR: Allergic rhinitis
ARIA: Allergic Rhinitis and its Impact on Asthma
ARs: Adverse reactions
DBU: Diagnostic biological unit
DF: *Dermatophagoides farinae*
PPT: *Dermatophagoides pteronyssinus*
EAACI: European Academy of Allergy and Clinical Immunology
ELISA: Enzyme-Linked Immune Sorbent Assay
EMA: European Medicines Agency
HDM: House dust mites
ITT: Intention to treat
LR: Local reaction
MedDRA: Medical Dictionary for Regulatory Activities
PP: Per Protocol
SAS: Statistical Analysis Software
SMs: Storage Mites
SPT: Skin prick test
SP: Safety population
SR: Systemic reaction
TSU: Treatment Standardized Unit

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Introduction

Respiratory allergy brings together a set of conditions with a highly health burden in the world (1, 2). Mites cause allergy disease in more than 10% of globe population and 90% of the people diagnosed from allergic asthma presents sensitization to domestic mites (3). House dust mites (HDM) are the most abundant aeroallergen in indoor environments, especially in warm and moist areas like Iberian countries (4).

Usually, mites are classified in two vast groups: HDMs belonging to the *Pyroglyphidae* family and storage mites (SMs) belonging to *Glycyphagidae* and *Acaridae* families (5). *Dermatophagoides pteronyssinus* (DPT) and *Dermatophagoides farinae* (DF) for HDM, followed by *Lepidoglyphus destructor* for SMs in a specific area of the north-west of Spain, were the most common species found in an epidemiologic study describing the prevalence of mites' sensitization in four different areas of the country (6). This geographical mites' distribution can imply different sensitization profile in patients of diverse areas and different allergen immunotherapy composition needs (3, 7).

It is well ascertained that allergy immunotherapy (AIT) is the unique available therapeutic option to target the disease and not only symptoms (8-11). Thanks to their disease-modifying effect, specific AIT gets immune system tolerance to clinically relevant allergens through triggering specific blocking antibodies, activating mediators and achieving the decrease of the inflammatory response in tissues. Probably, the AIT prescription is considerably lower than 10% of patients with AR or asthma (12, 13).

According to EMA guidelines, mixture of different allergenic sources is only recommended when they are taxonomically related (14). In the case of DPT and DF mixture, the similarity and cross-reactivity is so high, between 80-90 % (15, 16) that it could be enough to receive unique vaccine with one of these two allergen extracts. As a consequence of the enormous homology, any of the two allergenic sources could be proposed as the representative homologous specie (17).

However, a considerable number of clinicians remains prescribing HDM mixture (50% DPT and 50% DF) mainly based on patient's sensitization results (18,19).

Therefore, an open multicentre clinical trial in adult patients with allergic rhinitis (AR), (with or without asthma) using a standardized native depot HDM mixture extract for subcutaneous immunotherapy was conducted. The principal objective was to establish the tolerability and safety of an abbreviated treatment schedule in patients sensitized to HDM.

Materials and methods

Study design and ethical considerations

Five hospitals in Spain collaborated in this open, multicentre and phase I clinical trial. Likewise, the study was conducted in accordance with the principles of the Declaration of Helsinki and the ICH guideline on Good Clinical Practice. It was approved by relevant ethics committees and by the Spanish regulatory authorities, (EudraCT 2015-004712-38). Prior to their participation, written informed consent was given by every patient.

Study population

Patients had to meet the following criteria: age 18-60 years, clinical history of perennial AR due to HDM for at least 2 years prior to the study inclusion, a positive skin prick test to DPT or DF (wheal diameter ≥ 3 mm) and specific immunoglobulin E (sIgE) against DPT or DF levels ≥ 0.7 kUa/L determined by ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden). Results of SPT performed within 12 months prior to the inclusion were accepted. Only patients with concurrent mild asthma were allowed to participate.

The following were defined as exclusion criteria: to have received immunotherapy against HDM or a cross-reactive allergen in the 5 years prior the study inclusion, current administration of immunotherapy for any other allergen, moderate to severe asthma, forced expiratory volume in 1st second (FEV1) < 70%, clinically relevant perennial sensitization different of HDM. The following conditions were additional exclusion criteria: history of anaphylaxis, chronic urticaria, moderate to severe atopic dermatitis; immunological, cardiac, renal or hepatic diseases; current treatment with immunosuppressants, anti-IgE, tricyclic antidepressants, psychotropic drugs, beta-blockers, or angiotensin-converting enzyme inhibitors and pregnant or breast-feeding women.

Study interventions

Patients were treated with a native depot mixture of DPT and DF subcutaneous treatment, (Allergovac® Depot, ROXALL Medicina España S.A., Zamudio, Spain) consisting of two different strengths. The abbreviated build-up schedule comprised 6 doses at weekly intervals (± 2 days): 3 doses (0.2, 0.5 and 1 mL) from vial 2 (100 Treatment Standardized Units (TSU)/mL), and 3 subsequent administrations (0.2, 0.5 and 1 mL) from vial 3. The last dose of the increasing period, 1 mL of vial 3, 1000 TSU/mL, was the target maintenance dose and was administered at monthly intervals, during one trimester, being the whole treatment duration of 17 weeks. The concentration of the major HDM allergens for group 1 were: Der p1 0.44 µg/mL and 0.34 µg/mL Der f1 and for group 2 were Der p2 0.69 µg/mL and 0.45 µg/mL Der f2. Some dose schedule variations were allowed in the event of adverse reactions according to the standards for practical allergen-specific immunotherapy recommendations (20).

Outcome measures

In this study all adverse events (AEs) were registered for tolerability assessment. The primary outcome was the incidence of adverse reactions (ARs), recorded at participating sites during the

30 minutes after each vaccine administration. In addition, ARs were also collected by reviewing the patients' diaries designed to register any unpleasant experience outside immunotherapy units and by telephone calls. ARs were defined as all noxious and unintended responses to any dose of the investigational allergen vaccine administered. These reactions were classified as immediate (within 30 minutes after the vaccine administration) or delayed (> 30 minutes after vaccine administration).
In the same way, adverse reactions were classified as local (LR, reactions taking place at the arm where vaccine was administered), or systemic reactions (SRs, generalised symptoms taking place far away from the administration site). According to LRs extension, we consider clinically significant the immediate LR ≥ 5 cm and the delayed LR ≥ 10 cm or those implying a dose modification in the next administration. Additionally, LRs were described as diffuse inflammation, redness, erythema, local painfulness, pruritus or reaction in injection site (when two or more local symptoms took place simultaneously). SRs were classified by the investigators according to the European Academy of Allergy and Clinical Immunology EAACI guidelines (20) and also by the Medical Dictionary for Regulatory Activities (MedDRA).
Dose-response skin prick test (SPT) was performed using four increasing concentrations of HDM mixture extract (100, 1.000, 10.000 and 100.000 DBU/mL, Diagnostic Biologic Units) as well as positive (histamine 10 mg/mL) and negative (saline) controls. Titrated skin prick test for basal and final visits, were provided by ROXALL Medicina España S.A. to the study participants. The batch used for the whole study population was the same. The change in cutaneous reactivity (wheal area in mm^2) from baseline to the final visit was measured.
Regarding the immunological effects' assessment, serum samples were obtained at baseline and final visits to determinate immunoglobulin levels (sIgE, sIgG and sIgG4) against DPT and DF whole extract by ELISA (Enzyme-Linked Immune Sorbent Assay) as previously described in Sola J. *et al.* (2015) (21). Samples were frozen and sent to ROXALL's central laboratory for bioanalysis in accordance with Good Laboratory Practices.
Moreover, specific immunoglobulin titters IgE against Der p1, Der p2 and Der p10 were analysed at baseline and final visit by ImmunoCAP® (Thermo Scientific, Uppsala, Sweden).

Statistical methods

We described three populations: safety population (SP), patients who received at least one dose of treatment, intention-to-treat (ITT) population, patients who met all inclusion/exclusion criteria, received at least one dose of treatment, and had available data on subrogate efficacy variables, and the per-protocol (PP) population, patients who met previous criterial and moreover achieved their target maintenance dose and completed the study without any major protocol deviation.
Tolerability and safety were analysed using descriptive statistics. Categorical variables were described by absolute and relative frequencies and in continuous variables the mean and the standard deviation were applied.
Changes in immunoglobulin levels and SPT values from baseline to final visit, were analysed by means of the Wilcoxon non-parametric test for paired samples. A bilateral statistical significance level of 0.05 was displayed to all statistical tests. Statistical analyses were performed using the Statistical Analysis software (SAS) version 9.4.
Sample size was calculated considering a percentage of ARs of 22,9% (22). Establishing a confidence interval of 95% with a precision of ± 4 percentage unit and assuming a 5% of drop outs, the number of patients to provide adequate data on the primary endpoint was 42.

Results

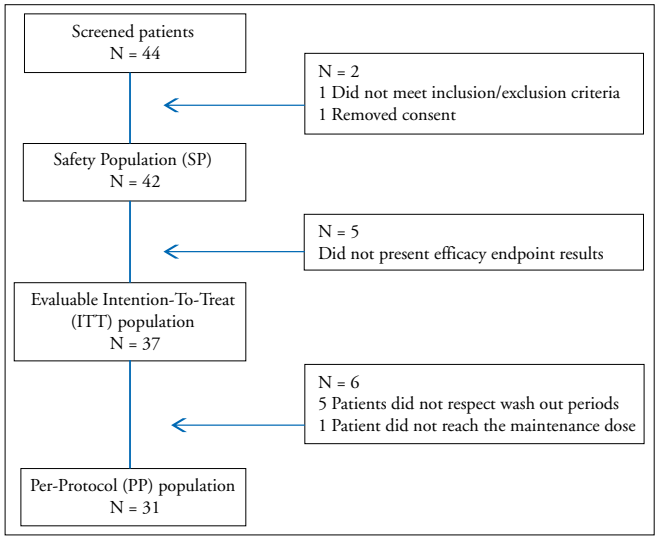
Descriptive data

A total of 44 patients were recruited from August 2016 to April 2017. Out of them, one was a screening failure and other one removed the consent prior to start treatment so, 42 patients were assigned to receive HDM mixture AIT and were analysed in SP. Additionally, in this study there were 6 early discontinuations: 1 due to AE, defined as hearing loss, 1 for surgery intervention, 3 for loss of follow up and 1 for change in the residence address. Rhinoconjunctivitis secondary to sensitization to *Dermatophagoides* was confirmed in each participant by allergy diagnostic tests and a rigorous clinical history. Regarding other sensitizations, the percentage of patients sensitized to grass pollen was 7.14 %, to weed pollen 4.76%, and to tree pollen 2.38%. However, these aeroallergen sensitizations were not clinically relevant or did not interfere with the collecting data period. ITT population included 37 patients since 5 were excluded due to the absence of data on immunoglobulins or dose-response SPT at final visit. Finally, 31 patients remained in the PP population, mainly as a result of major protocol deviations. Patient's distribution is shown in **figure 1**. Most patients showed sIgE class ≥ 4 against whole DPT and DF extract: 38.1% and 50% respectively. Subjects' baseline clinical characteristics and sIgE profile is presented in **table I**.

Tolerability and safety

All patients presented at least 1 AE in the study, being classified the majority of them as mild to moderate intensity. The most frequent reported AEs were, injection site reaction, headache, upper respiratory tract infections and digestive system disorders. All AE were non-serious and the vast majority were resolved with symptomatic medication.
ARs were summarized in **table II** and **III**. Out of 289 dose administrations, 6 (2.1 %), were considered as clinically relevant immediate LR, and 21 (7.3%) clinically relevant delayed LRs. All of them described as injection site reaction.

***Figure 1** - Study flow chart.*



Regarding systemic reactions, 22 SRs (7.6% of dose administrations) were recorded; six grade 0 (2.1%), fifteen (5.2%) grade I and one grade II (0.3%). There were no systemic reactions grades III or IV. All of them are described in **table III**.

SRs were resolved with symptomatic treatment or a change in the next administration dose. All patients recovered of the ARs at the end of the study. One patient in spite of the dose modifications performed in the schedule, did not reach the maintenance dose established in the study protocol due to adverse reactions.

This patient with the worst systemic reaction, described as delayed rhinitis with dyspnoea responded to the symptomatic treatment with beta 2 blockers, inhaled corticosteroids and antihistamines. At basal period, she/he presented class 4 levels of sIgE against DPT and DF. However, papule areas before immunotherapy treatment showed a size similar to the mean of the sample population.

Clinically relevant changes in blood count and biochemistry parameters were not observed in any patient after receiving immunotherapy treatment.

Immunoglobulin levels

Statistically significant increases in serum sIgG and sIgG4 titters against DPT and DF whole extract at final visit were observed compared with basal visit (both $p < 0.0001$; Wilcoxon test). Serum sIgE levels to DPT and DF slightly decreased at final visit, achieving statistical significance ($p < 0.0002$; Wilcoxon test) (**figure 2**). On contrary, a statistically significant increases in serum sIgE against Der p1 and Der p2 at final visit were observed in comparison with basal visit ($p < 0.0001$ and $p < 0.02$, respectively; Wilcoxon test). As it was expected, these results were maintained in PP population.

***Table I** - Patients' baseline clinical characteristics.*

Baseline characteristics	
Number of patients *(SP)*	*42*
Age *(years), mean ± (SD)*	*33.6 ± 9.1*
Women *n (%)*	*21 (50.0)*
Race *n (%)*	
Caucasians	*31 (73.8)*
Hispanics	*7 (16.7)*
Arabs	*2 (4.8)*
Asians	*2 (4.8)*
Rhinitis ARIA classification (32)	
Intermittent mild n (%)	*1 (2.4)*
Persistent mild n (%)	*9 (21.4)*
Intermittent moderate-severe n (%)	*2 (4.8)*
Persistent moderate-severe n (%)	*30 (71.4)*
Main concomitant allergic condition	
Asthma n (%)	*7 (16.7)*
Time from diagnostic *(years), mean ± (SD)*	*4.7 ± (8.4)*
(BMI), Kg/m² *mean ± (SD)*	*24.7 (4.85)*
Vital signs *mean ± (SD)*	
Systolic blood pressure, mmHg	*114.7 (14.1)*
Diastolic blood pressure, mmHg	*71.7 (10.6)*
Heart rate, bpm	*71.6 (9.2)*
sIgE DPT CAP class *n (%)*	
2	*1 (2.4)*
3	*9 (21.4)*
4	*16 (38.1)*
5	*16 (38.1)*
sIgE DF CAP class *n (%)*	
2	*1 (2.4)*
3	*11 (26.2)*
4	*21 (50.0)*
5	*9 (21.4)*

(SP) safety population, (SD) standard deviation, (BMI) Body Mass Index, (mmHg) millimetres of mercury and (bpm) beats per minute.

Cutaneous reactivity

Mean values of wheal area in mm^2 were significantly reduced at final visit compared with baseline in each one of the four tested vials against HDM mixture (**figure 3**). Moreover, a statistical significance was achieved with all tested vials ($p < 0.04$; Wilcoxon test from vial 1 to vial 4). These cutaneous results were also reproduced in the PP population.

Discussion

Despite the fact of great homology between DPT and the rest of the mites belonging to the family *Pyroglyphidae,* (15, 16, 23)

Table II *- Summary of adverse reactions in SP.*

	Schedule Phase	**Patients number n (%)** *42 (100%)*	**Administered doses n (%)** *289 (100%)*
Local reactions		*37 (88.1%)*	*187 (64.7%)*
Clinically relevant immediate LRs	*Initiation Phase*	*5 (11.9%)*	*6 (2.1%)*
Clinically relevant delayed LRs	*Initiation Phase*	*12 (28.6%)*	*21 (7.3%)*
Systemic reactions		*14 (33.3%)*	*22 (7.6%)*
Grade 0	*Initiation Phase*	*6 (14.3%)*	*6 (2.1%)*
Grade I	*Initiation Phase*	*9 (21.4%)*	*15 (5.2%)*
Grade II	*Initiation Phase*	*1 (2.4%)*	*1 (0.3%)*

n (%) number and percentage of adverse reactions, LR (local reaction) and SP (safety population).

Table III *- Description of systemic adverse reactions by administration doses.*

(N = 289 doses administered)

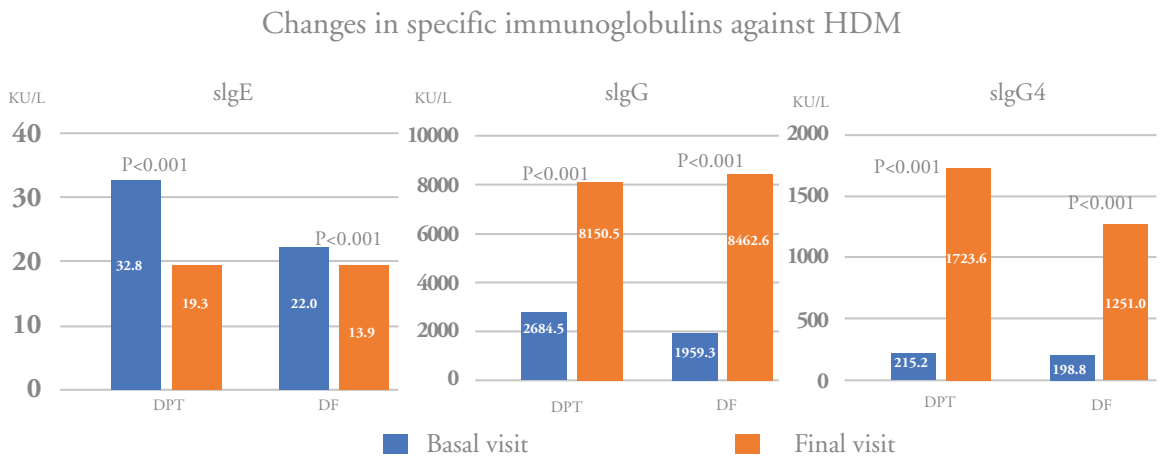
	n (ARs) (%)	**Number**	**Description**
Grade 0	*6 (2.1%)*	*2*	*Headache*
		3	*General desconfort + nausea + dizziness*
		1	*Non-specific cough*
Grade I	*15 (5.2%)*	*1*	*Conjunctivitis*
		1	*Dermatitis*
		4	*Allergic rhinitis*
		2	*Urticaria*
		1	*Generalized pruritus*
		1	*Pruritus out of the injection site*
		2	*Erythema out of the injection site*
		1	*Throat irritation*
		1	*Pharyngitis*
		1	*Allergic cough*
Grade II	*1 (0.3%)*	*1*	*Rhinitis + dyspnea*

n (%) number and percentage of adverse reactions. (ARs) Adverse Reactions.

allergy clinicians commonly prescribe mixed vaccines to treat patients polysensitized to mites. As it was mentioned, the most common mites with positive results in diagnostic prick tests performed in patients with respiratory allergy in Spain, were DPT, DF and *Lepidoglyphus destructor* (6), excluding the islands. As a consequence, immunotherapy containing a mixture of DPT and DF is frequently prescribed, although a recent publication did not find differences in efficacy between two commercial mites' extracts: one with DPT as single source and other with a mixture of DPT and DF (50/50) (24).

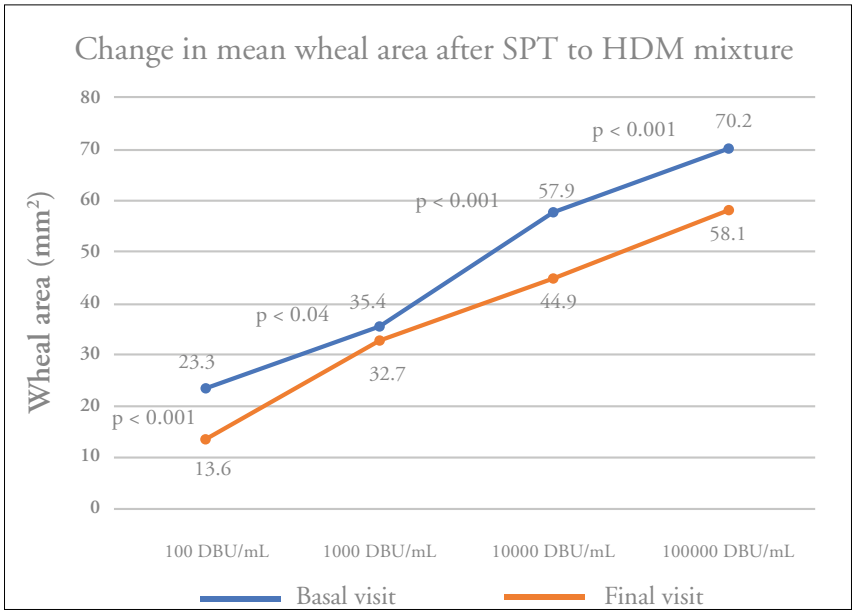
ROXALL Medicina España S.A. (formerly Bial) conducted two clinical trials with Allergovac® Depot native DPT 100%. A placebo-controlled Phase I study to evaluate three different build-up schedules (22) and a dose finding randomized controlled trial to compare the efficacy of five different doses (25). In both studies, the tolerability profile of the abbreviated schedule could be ascertained as good. However, this evidenced data must be interpreted with caution when they are extrapolated to another marketed product with HDM mixture. A new safety and tolerability trial, with the same schedule and a treatment containing

Figure 2 *- Changes in specific immunoglobulins against HDM.*



Changes in specific immunoglobulins against HDM. Corresponding p values according to Wilcoxon test are indicated.

Figure 3 *- Change in mean wheal area after SPT with HDM mixture.*



Change in mean wheal area after SPT with HDM mixture at final visit versus baseline. p values according to Wilcoxon test are indicated.

a DPT/DF mixture (50/50), was designed in order to avoid this extrapolation.

The percentage of systemic ARs with the vaccine under study was slightly higher than with the DPT 100% vaccine used in previous ROXALL clinical trials. In current study, a 7.6% of systemic ARs was described versus 4.8 % in the phase I study (22) and 3.8% with the group containing the commercial dose, in the dose finding trial (25). In comparison with other similar marketed products, the encountered results are in a similar range, thus a study conducted by Tabar *et al.* (26) showed a systemic ARs percentage of 8.8% by patient. In another clinical trial with a DPT 100% formulation, a 9.8% of ARs was report-

ed (27). In a comparative study of two schedules with HDM depot native immunotherapy (28), the percentage of ARs with conventional schedule reached 13.8% and 10.7% with cluster scheme. Additionally, in similar designed studies, but with different extracts composition apart from HDM, the percentage of ARs was even higher reaching in some cases 21% (29).

This depot formulation induced an early immunological response, confirmed by statistically significant increments of sIgG and sIgG4 levels against DPT and DF, after approximately 3 months of therapy. Similar results could be observed in other studies, where a fast increase in sIgG and sIgG4 can be associated with the effect of blocking IgE-binding to allergens and immune response modification (26, 30, 31). These results are in line with the immunologic and skin prick test outcomes observed in previous studies with DPT 100% (Allergovac® Depot, ROXALL Medicina España S.A., Zamudio, Spain) (22, 25). Regarding sIgE determination against Der p1 and Der p2, surprisingly a statistically significant increased at final visit was observed. These increases could be attributed to the effect of other allergens different to Der p1 and Der p, with relevance in the study patients' immune response.

Considering the cutaneous reactivity to the causal allergens, a statistically significant reduction in immediate skin response to the different concentrations of DPT and DF combination was observed, showing a decrease in the mean papule size produced by each concentration tested. This result is in the line of another clinical trial with an extract of HDM mixture after a short administration of specific immunotherapy (26).

Conclusions

Given the heterogeneity in participants, allergens, schedules, dosing treatment and adverse reactions reporting methodology, it is difficult to compare tolerability results between different available studies. However, this clinical trial shows that the assayed abbreviated schedule with native depot HDM mixture, (Allergovac® Depot ROXALL Medicina España), has an acceptable tolerability profile. Moreover, preliminary positive efficacy response can be observed due to a significant immunological and cutaneous reactivity changes in subjects suffering from allergic rhinoconjunctivitis. These promising results should be worth to be confirmed in a larger controlled clinical trial.

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Conflict of interests

Ignacio Antépara has received research fees from, Novartis, GSK, Astra-Zeneca, Sanofiand Bial. Albert Roger has received research fees from Roxall, Allergy Therapeutics, Stallergenes, Leti, Hal, Merck, Diater. Consultant fees: Allergy Therapeutics, Stallergenes, Merck. Speaker fees: Roxall, Allergy Therapeutics, Leti, Merck. Nagore Bernedo has received consultant fees from ALK and Allergy Therapeutics, speaker fees from Roxall and research fees from Merck. Fernando Rodríguez has received research fees from GSK, ALK and Roxall. Speaker fees: GSK, Novartis, Astra-Zeneca and Chiesi. Begoña Madariaga, Juan A Asturias, Leire Begoña, Alberto Martínez, Araitz Landeta and María C Gómez are fulltime employees of ROXALL Medicina España S.A. Ramón Lleonart has no conflicts of interest to declare. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Characteristics of patients with C1 esterase inhibitor deficiency: a single center study

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Key words

Hereditary angioedema; C1 esterase inhibitor deficiency; hereditary angioedema type 1; C1 esterase inhibitor; familial mediterranean fever.

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Summary

Hereditary angioedema (HAE) is a primary complement factor deficiency, characterized by recurrent submucosal/subcutaneous swelling episodes. SERPING1 gene defects encoding C1 esterase inhibitor (C1INH) are responsible from the disease.

Fifteen patients with HAE are retrospectively evaluated in this study. All patients (n = 15) had HAE type I, 13 were from the same family, other two from two different families. Median age at onset of symptoms was 7 years (2-20); median age on diagnosis, 12 (0,5-41) and median delay in diagnosis, 3 years (0-33). Clinical symptoms were extremity edema(92,3%), facial edema(46%), abdominal pain (46%), genital edema (46%), and laryngeal edema (23%). Some patients suffered from recurrent abdominal pain, had been empirically given colchicine with familial mediterranean fever (FMF) when they admitted. One presented with bullous skin eruption, soon after developed extremity edema. Both resolved spontaneously after C1INH concentrate therapy. Two females suffered from recurrent genital edema after sexual activity. One patient experienced compartment syndrome-like swelling of extremity after playing football. One patient diagnosed with panic attack due to fear of death by asphyxiation, and was diagnosed with HAE disease. A nonsense mutation in exon 8, a missense mutation in exon 2 in SERPING1 gene was present in Family 1 and another patient (P14) from the other family, respectively. Sporadic/autosomal dominant inheritance ratio was 2/3 in the families present in our series.

Patients with HAE presents with a large spectrum of symptoms. In mediterranean countries, patients with abdominal attacks may be misdiagnosed with FMF. Thus, health-care professionals should be alert, and put HAE in the first line of differential diagnoses when the disease symptoms are present. Consequently, morbidity/mortality will decrease with effective treatment options.

Introduction

Hereditary angioedema (HAE) is an autosomal dominant disease that occurs due to the mutations in SERPING1 gene encoding the serine protease C1 esterase inhibitor (C1INH). It is characterized by recurrent episodes of submucosal or subcutaneous swelling, most often affecting skin or mucosal tissues. The more common form, HAE type I, (85% of patients) results from C1INH deficiency and shows low C1INH function, while HAE type II is caused by C1INH dysfunction with normal or elevated C1INH levels (1). In HAE type III, there is no change in C1INH, and the disease includes patients with FXII, plasminogen and angiopoietin-1 gene mutations, as well as cases with unknown causes.

The diagnosis of HAE is based on clinical history, physical findings during episodes, a family history of angioedema, and analysis of C1INH concentration and activity in plasma. Genetic analysis is also necessary to make a definite diagnosis of HAE. The treatment is based on prevention of the attacks, reduction of morbidity and mortality and improvement in life quality. The gold standard treatment for acute attacks is plasma derived nanofiltered C1INH concentrate. Recombinant C1INH concentrate, icatibant (bradykinin B2 receptor inhibitor) and ecallantide (kallikrein inhibitor) is also used for acute attacks. Attenuated

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androgens and plasmins are highly effective despite the side effects and they are the mainstay of the long term prophylaxis.
The aim of this study is to investigate the characteristics, clinical and laboratory findings of patients with HAE.

Patients and methods

Patients who were diagnosed with HAE during 2009-2019 period in pediatric immunology department are enrolled into the study. The clinical and laboratory findings retrospectively reviewed from the files of the patients.
The level and the activity of C1INH protein were recorded. The genetic defect was analyzed by the help of primary immunodeficiency diseases (PID) next generation sequencing (NGS) panel (2).

Results

There were totally 15 patients, 13 from the same family (**figure 1 A**). P14 and P15 are from different families. All patients had HAE type I. Demographic, clinical and laboratory characteristics of the patients are given in **table I**. The median age at onset of symptoms was 7 years (2-20). The median age on diagnosis was 12 (0.5-41). The median delay in diagnosis was 3 years (0-33).
The most common clinical symptom was extremity edema (92,3%). Facial edema (46%), abdominal pain (46%), genital edema (46%) and laryngeal edema (23%) were the other most frequent symptoms. The proband in Family 1, P1 (38 year-old), first admitted to our clinic with facial edema after trauma. After he was diagnosed with HAE, the pedigree was overviewed for the analysis of the family members with similar complaints.
The father of P1, P2 (53 year-old) had recurrent laryngeal edema. P2's brothers P3 and P4, and P4's doughter, P5, had suffered from recurrent abdominal pain for years. They were given colchicine empirically for recurrent abdominal pain with the possible diagnosis of familial mediterranean fever (FMF). MEFV analysis was performed to P4 and P5, and found to be normal. Upon low C1IHN level, he and his daughter were diagnosed as HAE type I respectively. P1's doughters, P6, 9, 10, P1's brother P7 and his son, P1's sister P8 and her children P12 and P13 were diagnosed with HAE type I.
P1, P2, P3, P6, and P7 were suffering from recurrent laringeal edema. P2 and P3 had recurrent abdominal pain for 25 and 33 years, respectively. P1, P7 and P8 had recurrent peripheral edema. P6,9,10,12, and 13 were diagnosed before symptoms occured. Despite low C1INH levels, P12 and P13 are asymptomatic. Once P7 presented with bullous skin eruption in the distal part of right upper extremity occurred as an early symptom of angioedema, resolved spontaneously after therapy (**figure 2**). P5 and P8 had suffered recurrent genital edema after sexual activity. A compartment syndrome of right leg developed in P6 after playing football, responded to medical therapy.
Molecular analysis of Family 1 was a previously defined nonsense mutation in SERPING1 gene (c.1450 C > T (p.Gln484Ter) (**figure 1 B**) (3). P14 was diagnosed when she was admitted to hospital with extremity edema at the age of 2,5 years. Low complement C4 and C1INH level was observed. Mutational analysis demonstrated a novel missense mutation in SERPING1 gene by NGS panel (c.5C > T (p.Ala2Val) (**figure 1 A**)). P15 suffered from recurrent skin and laringeal edema after the age of ten. He admitted to hospital with the complaint of hard breathing and he was diagnosed as panic attack. He was diagnosed as HAE with 9 years delay.
All patients have low serum C1INH and complement C4 levels that compatible with HAE type I. Mean serum levels of C1INH was 7,0 ± 2,7 mg/dl (normal range: 15-35 mg/dl) ranging from

Figure 1 - (A) *Pedigree of the Family 1.* ***(B)*** *The position of the genetic defects.*

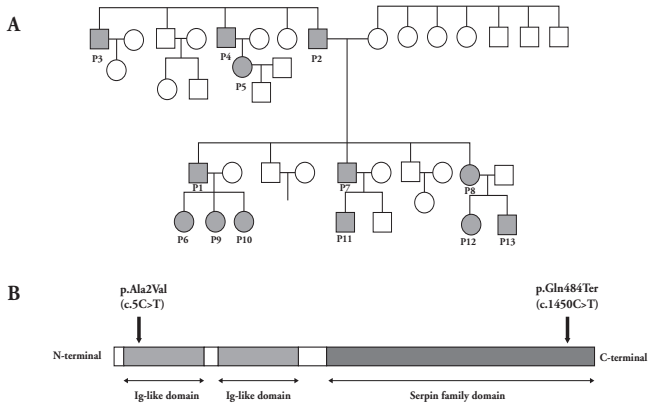

Figure 2 - *Bullous skin eruption in the distal part of right upper extremity of P6, occured one time before extremity angioedema.*

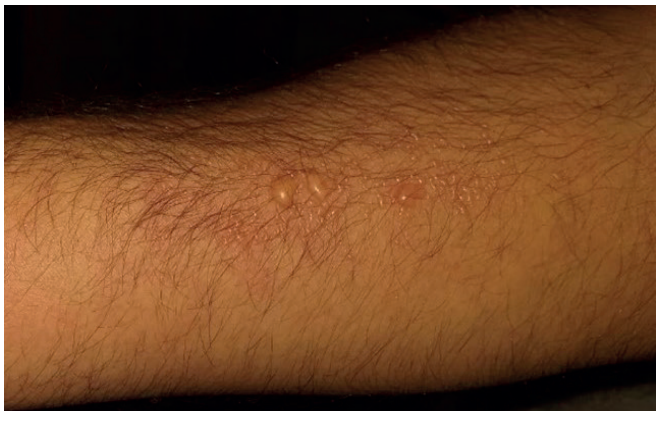

Table I *- Demographic, clinical and laboratory features of patients.*

Patient No	Gender (M/F)	Age	Age at onset of symptoms	Age at diagnosis	Sites involved during angioedema attacks	Treatment	Severity of disease	C4 (mg/dl) (16-38 mg/dl)	C1INH (mg/dl) (15-35 mg/dl)
P1	M	39	20	23	Extremities, abdominal, genital	Danazol	Mild	5	3,5
P2	M	60	8	41	Extremities, abdominal	C1INH concentrate (during attacks)	Mild	4,7	2,8
P3	M	51	7	32	Extremities, facial, laringeal, genital	C1INH concentrate (during attacks)	Severe	9,2	7,2
P4	M	53	10	34	Extremities, facial, genital		Mild	3,2	8,8
P5	F	25	7	12	Extremities, facial, abdominal, genital	C1INH concentrate (during attacks)	Severe	4,6	10,2
P6	F	11	5	1	Extremities, abdominal	Tranexamic acid	Mild	5,5	4,3
P7	M	41	10	22	Extremities	Danazol	Mild	5,3	4,9
P8	F	32	18	20	Extremities, facial, laringeal	Tranexamic acid	Mild	3,4	5,9
P9	F	10	7	2	Abdominal	C1INH concentrate (during attacks)	Moderate	10	6,5
P10	F	3	2	0,5	Ekstremities	C1INH concentrate (during attacks)	Mild	5,6	11
P11	M	10	5	5	Extremities, facial	Danazol	Mild	8,7	8,5
P12	M	1,5	No symptoms developed	1			Asymptomatic	3,4	7,4
P13	F	5	No symptoms developed	2			Asymptomatic	3,8	6,9
P14	F	14	2,5	2,5	Extremities, abdominal		Moderate	5,6	5,3
P15	M	20	10	19	Extremities, laringeal, genital		Mild	6	12,4

2,8 to 12,4. Because of having multiple attacks, danazol was started to P1 and P7, and tranexamic acid was started to P6 and P7. No patients had angioedema attacks after prophylaxis treatment. P4 refused the treatment. P2, P3, P5, P9 and P10 receive C1INH concentrate when attacks occur, and refused to receive long term prophylaxis treatment due to side effects. P12 and P13 are asymptomatic.

Discussion

HAE is characterized by recurrent episodes of submucosal or subcutaneous swelling, most often affecting skin or mucosal tissues and developing after trauma. In some cases clapping of hands, prolonged sitting on a hard surface may cause an attack. In this study angioedema occured in 13 out of 15 patients. P6 was suffered from compartment syndrome of the leg and improved with medical therapy, however in some cases fasciotomy might be necessary (4). Bullous skin eruption, which was reported before in HAE (5), also developed once in P6 before extremity angioedema. As it resolves spontaneously, there is no need for diagnostic workup and treatment. Other rare clinical presentations were previosly reported such as transient ischemic attack symptoms due to local cerebral edema and reduced cerebral perfusion, recurrent episodes of pancreatitis due to pancreatic edema and pancreatic duct obstruction, hypovolemic shock due to abdominal ascites, tetany due to hyperventilation during the abdominal pain, hemorrhagic diarrhea due to massive bowel edema, hematuria due to submucosal edema of vesical walls (6-8).

HAE is a rare disease, and most of the patients are diagnosed years after onset of symptoms. P4 and his doughter were first misdiagnosed as having FMF and administered colchicine treatment for years. They didn't benefit from treatment as expected. The transient edema of the bowel wall may cause gastrointestinal pseudoobstruction, and is generally characterized with ab-

dominal pain, vomiting and diarrhea. Bork *et al.* reported that 28% of patients presented with abdominal attacks long before they noted swelling of their skin (7). For this reason, abdominal angioedema without skin swelling may be misdiagnosed as FMF, in FMF endemic countries, like Turkey. FMF is an autosomal recessive disorder characterized with recurrent fever, serositis, erysipelas like skin rash in the lower extremities and elevated acute phase reactants. Most of the patients have biallelic MEFV gene mutation, and achieve complete remission with cochicine (9). Thus, the physicians should suspect about the diagnosis of HAE, unless the patients diagnosed previously with FMF respond to cochicine therapy.

P15 admitted to emergency department with the complaint of dyspnea, he was misdiagnosed as panic attack and received psychiatric treatment. Laryngeal attacks not only cause fear of death by asphyxiation, but also lead to depression and anxiety. On the contrary, depression and anxiety may trigger initiation of attacks. Therefore HAE should be considered as a disease having a psychosocial dimension. Misdiagnoses of HAE according to symptoms are shown in **table II** (10, 11).

In this study, the laboratory workup of all patients were consistent with HAE type I, low C4, C1INH level, and C1INH function were recorded. After the proband was diagnosed, the other 13 patient were diagnosed subsequently. Thus, five family members had a chance of having diagnosis before the onset of symptoms due to the autosomal dominant inheritance pattern of the disease.

A nonsense mutation and a missence mutation in SERPING1 gene were found by the help of NGS in the first family and P14, respectively. Previously Kesim *et al.* reported an initial codon change in exon 2 and a nonsense mutation and 9-bp deletion in exon 8 (12). Although first family has the same mutation, the onset of the symptoms, the tissues affected (skin,mucosa, larynx, abdomen, extremities) and the severity of the disease among family members are different, showing that there is no genotype-phenotype relationship. P14 and P15 were thought to be sporadic cases as there was no family history. The prevelance of the HAE is known to be about about 0,2-1/10000 (12, 13). However, the ratio of sporadic and AD inherited cases to all cases is unknown. Previously Pappalardo suggested that 45 out of 137 patients diagnosed with HAE in his series were sporadic (14). According to this study, 2/3 of the cases have AD inheritance pattern, and 1/3 of the cases are sporadic. This ratio was 1/3 in our series. Among three families, AD inheritance was present in Family 1, and other two patients were sporadic cases. So, genetic counseling and information about the preimplantation genetic study should be offered to cases, except sporadic ones. As soon as patients are diagnosed with HAE, they have access to effective treatment options. Consequently, morbidity and mortality decreases.

Table II *- Misdiagnoses in HAE according to symptoms (10, 11).*

Symptoms	Differential diagnoses
Laryngeal edema	Anaphylaxis Tonsillitis Panic attack
Lip edema	Cheilitis granulomatosa Melkersson-Rosenthal syndrome
Facial edema	Superior vena cava syndrome Autoimmune conditions (SLE, polymyositis, dermatomyositis, Sjogren syndrome) Allergic contact dermatitis
Pretibial mixedema	Thyroid disorders
Extremity edema	Autoimmune conditions (SLE, polymyositis, dermatomyositis, Sjogren syndrome) Allergic contact dermatitis Compartment syndrome
Abdominal edema	Familial mediterranean fever Appendicitis GERD Irritable bowel disease Peptic ulcer Endometriosis
Genital edema	Orchitis

GERD, gastroesophagial reflux disease; SLE, systemic lupus erithematosis.

The gold standard treatment for acute attacks is plasma derived nanofiltered CINH concentrate. Recombinant C1INH concentrate, icatibant (bradykinin B2 receptor inhibitor) and ecallantide (kallikrein inhibitor) are also used for acute attacks (15). Because of being recently available in Turkey, we didn't have opportunity to use these therapies for the treatment of acute attacks. Anabolic steroids are the mainstay of HAE prophylactic treatment of, however side effects like hepatoma and virilization in women may cause discontinuation of therapy. Additionally, the risk of growth retardation restricts its use in children. Plasmin inhibitors are less effective than anabolic steroids, but are preferred in children and women in longterm prophylaxis after the evaluation for the risk of thrombosis. Some of our patients refused the prophylaxis of danazol due to concerns about the side effects, such as hepatic toxicity and hepatic disease. They may be preferred in adult male patients. Regular control for liver disease is needed. However, others benefited from danazol and tranexamic acid used for long term prophylaxis, and after treatment no attacks and side effects were recorded. Other long term prophylaxis options, such as lanadelumab is not available in Turkey and

C1INH concentrate for long term prophylaxis treatment is not cost effective. Fresh frozen plasma is safe and effective for acute exacerbations of HAE, and can be used when C1INH concentrate can not be obtained (15). However improvement with FFP is slower. Although we used FFP with success, Nzeako *et al.* suggested that tissue swelling may increase with FFP as it involves other complement factors and kinins (16).

Conclusions

The diagnosis of HAE is based on clinical history, physical findings during episodes, a family history of angioedema, and analysis of C1INH concentration and activity in plasma. Anabolic steroids and plasmin inhibitors are effective for long term prophylaxis, but require close monitoring for side effects. There is a need to raise awareness of HAE among clinicians as the patients may be misdiagnosed as FMF, or panic attack. The health care professionals should consider that laryngeal attacks not only cause life threatening asphyxiation, but also have a psychosocial dimension. Genetic counseling and preimplantation genetic testing should be offered to patients except sporadic ones.

Author's contributions

Deniz Çağdaş, İlhan Tezcan and Elif Soyak Aytekin contributed to follow-up of patients. Elif Soyak Aytekin involed in collecting data and samples. Elif Soyak Aytekin and Deniz Çağdaş contributed in writing the manuscript. Elif Soyak Aytekin, Deniz Çağdaş, and İlhan Tezcan involved in the analysis and interpretation of the findings. Cagman Tan did the molecular genetic studies. Deniz Çağdaş and İlhan Tezcan reviewed the manuscript. All the authors read and approved the final draft of this manuscript.

Conflict of interests

The authors declare that they have no conflict of interests.

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Pediatric urticaria in the Emergency Department: epidemiological characteristics and predictive factors for its persistence in children

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Acute urticaria; children; trigger factors; epidemiology; severity.

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Summary
Introduction. *Acute urticaria (AU) in children is a common clinical manifestation responsible for admission to the emergency department (ED). We aimed to analyze the epidemiological characteristics of AU in children and to identify predictors of both severity and progression.* ***Materials and methods.*** *We evaluated 314 children admitted to the ED with a diagnosis of AU. We analyzed information concerning its onset, duration, severity, possible triggering factors, and the persistence of symptoms after 1, 3, and 6 months.* ***Results.*** *The most common etiological factors were infections (43.9%); in up to 32.4% of cases, AU was considered as idiopathic. AU was significantly most common in males and pre-school children. At the 6-month follow-up, 9.5% of children presented a persistence of urticaria, mainly those with contact (44.4%) or idiopathic (30.4%) forms.* ***Conclusions.*** *The AU etiology identified by history in the ED may be a significant predictor of persistence after a first attack of AU.*

Introduction

Acute urticaria (AU) is a common skin disorder characterized by itching, wheals, and/or angioedema with a duration < 6 weeks. Urticaria is considered chronic when it is recurrent, with signs and symptoms recurring most days of the week, for six weeks or longer (chronic urticaria, CU) (1, 2). AU is reported in childhood (3.4% UK, 4.4% Germany, 5.4% Denmark), and its persistence is even less probable (0.1%-0.3%) (3). The first treatment of urticaria is the elimination of any identified trigger factors and then the use of second-generation antihistamines and corticosteroids (2). A detailed history of the factors that may predict the time and the severity of urticaria will help physicians to perform an appropriate clinical assessment. Several studies described the demographics and the etiologies of AU in children (4, 5), but the factors that may influence its duration and severity have not been well addressed.
Therefore, this study aimed to evaluate the prevalence of urticaria in children referred to Emergency Departments (ED) in Italy and to analyze the factors that may predict the duration of the first attack.

Materials and methods

We performed an observational clinical study on children with AU referred to ten Italian EDs from 1st October 2016 to 1 De-

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cember 2017. The Local Ethics Committees approved the study protocol, and the work was conducted in compliance with the Institutional Review Board/Human Subjects Research Committee requirements and with the Declaration of Helsinki and the Guidelines for Good Clinical Practice criteria.

Population

Inclusion criteria: patients of both sexes and < 18 years old, with a diagnosis of AU in agreement with international guidelines (2) and with no pharmacological treatment before ED evaluation, whose legal guardians signed the informed consent. Exclusion criteria: patients with CU or affected by autoimmune diseases or in treatment with corticosteroids; children whose legal guardians did not sign the informed consent.

Endpoint

The first endpoint was the evaluation of the characteristics of children with AU. The second endpoint was to determine the factors that could be used to predict the severity and duration of an initial episode of AU in children.

Experimental protocol

The diagnosis of AU and its management was made in agreement with the European Academy of Allergy and Clinical Immunology (EAACI) international guidelines (2). Children who satisfied the inclusion criteria were evaluated at 1, 3, and 6 months after the ED admission in order to obtain data on the efficacy of treatment and persistence of symptoms. We used a questionnaire *ad hoc* that included questions concerning the onset of the acute attack, its duration and severity, and the possible triggering factors. The same questionnaire was repeated at follow-up. The etiological diagnosis was mainly carried out through history taking and physical examination. Laboratory investigations were performed based on history and physical examination to identify the underlying cause. The disease activity was assessed with the weekly Urticaria Activity Score (UAS7) score (2).

Statistical analysis

All data were analyzed using the SPSS statistical program (Microsoft, Redmond, WA, USA) by evaluating the arithmetic characteristics, *e.g.*, mean, geometric mean, standard deviation (SD). Data parameters were checked for normality using the Shapiro-Wilk normality test. Data were analyzed by one-way ANOVA analysis of variance and the χ^2 test. Pearson's test was used to evaluate the correlation between urticarial and patients' characteristics. The threshold for statistical significance was set at $^*p < 0.05$. SPSS (SPSS Inc., Chicago, USA) software was used for statistical analyses. We defined and labeled this study as exploratory; therefore, we did not perform a power calculation.

Results

During the study, 314 children (148 females, 47.1%, and 166 males, 52.9%) aged < 17 years (median 70 months, range 2-442 months) with a diagnosis of AU that fulfilled the inclusion criteria were enrolled. AU was more common in males than in females ($p < 0.05$), and in the age range of 0 to 5 years (50.3%). Clinical evaluation and laboratory tests documented that the most common forms of AU were para-infectious (43.9%) and idiopathic (32.4 %) (**table I**). In particular, the idiopathic form was most common in children 6-10 years old ($p < 0.05$) and the infectious form in children under five years (**table II**). Moreover, the frequency of AU was significantly lower ($p < 0.01$) in children older than ten years compared to the younger ones.

Mild urticaria was diagnosed in 40.4% enrolled children, moderate urticaria in 44.5%, and severe urticaria in 14.9%.

The correlation between the severity of AU and the age of children, sex, etiology, and family history for allergic diseases is shown in **table III**. A positive family history for allergy was found in 114 children (36.3%), and it was significantly more frequent in those with AU induced by reactions to food (n = 24, 54.5%; $p < 0.01$), drugs (n = 5, 45.5%; $p < 0.05$) and contact (n = 8, 44.4%; $p < 0.05$) compared to children with idiopathic (n = 31, 30.4%) or infectious (n = 46, 33.3%) urticaria.

Drug treatment was given in 290 children (92.4%), and most commonly used drugs were antihistamines ($p < 0.01$) in monotherapy (n = 166; 57.3%) or with corticosteroids (n = 110; 37.9%).

During follow-up, 10.8% of children presented a recurrence of urticaria in the first month, 11% at three, and 9.5% at six months. The factors associated with the recurrence of AU are reported in **table IV**. Severity, sex, and familiarity did not seem to correlate with the persistence of urticaria. The ages that had had a greater tendency to CU (in particular at six months) were 5-10 years old and 10-15 years old. In particular, urticaria recurrences were most common in the contact (44.4%) and idiopathic (30.4%) forms without differences during the follow-up (**figure 1**).

Discussion

In this study, we analyzed epidemiologic data of children with AU admitted to ED; also, we evaluated several significant factors that may predict the severity of an initial episode of AU in children and its progression to a chronic form. AU is a common cause of admission of children to the ED, and it is estimated to affect 15%-25% of people at some point in their life (4, 6), commonly adult females (7, 8). In a register-based study, Ghazanfar *et al.* (9) recently documented that women were more frequently diagnosed with urticaria than men, probably because men are less likely to seek medical attention than women. In contrast, in our study, urticaria was more common in males and

Table I *- Clinical characteristic of urticaria in children enrolled in the study.*

Urticaria	Total	Male	Female	%
Idiopathic	101	48	53	32.4%
Infectious	139	75	64	43.9%
Food	43	27	16	14.0%
Drugs	12	9	3	3.5%
Poison	1	/	1	0.3%
Contact	18	7	11	5.7%

Table II *- Difference in the age of children with urticaria enrolled in the study.*

Urticaria	Total children (n)	Mean age (n)			
		0-5 years	6-10 years	11-15 years	16-18 years
Idiopathic	101	38	42	20	1
Infectious	139	87	37	13	2
Food	43	21	11	9	2
Drugs	12	5	3	4	0
Poison	1	0	0	0	1
Contact	18	7	4	5	2

Table III *- Relationship between gravity of urticaria and sex, age, etiology, and familiarity for allergy.*

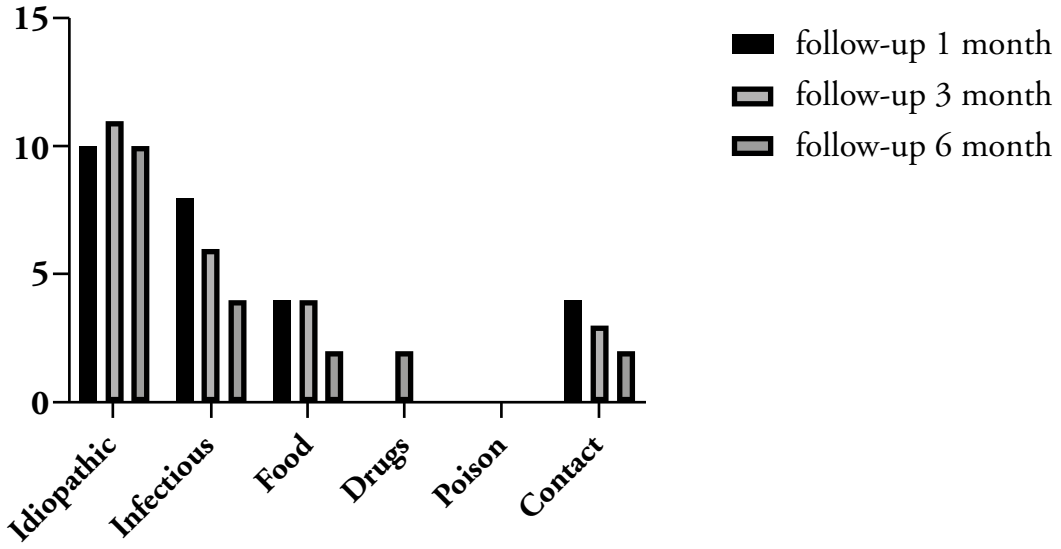
GRAVITY				
		MILD n.127 (40.4%)	MODERATE n.140 (44.5%)	HIGH n.47 (14.9%)
Physician-diagnosed cause of urticaria	Idiopathic	46	39	16
	Infectious	55	70	14
	Food	18	14	11
	Drugs	6	5	1
	Poison	0	1	0
	Contact	2	11	5
Sex	Female	63	62	23
	Male	64	78	24
Age	0-5 years	76	66	16
	6-10 years	33	52	12
	11-15 years	16	18	17
	16-18 years	2	4	2
With family allergic history		43	51	20

in pre-school children suggesting that probably age-related sex hormones can play a role in the pathogenesis of urticaria.
Previous studies reported that infections are involved in the development of AU in children (3-5, 10). Infections were the most common potential triggers of attacks of AU in the present study, occurring in 43.9% of the patients and mainly represented by upper respiratory tract infections. The observed frequency rate is similar to that reported in other studies (11, 12).
Bacterial infections of the teeth and the tonsils (*e.g.,* with streptococci) and gastrointestinal infections (*e.g., Helicobacter pylori* infection) have been described as potential triggers of AU. Nevertheless, the exact role and pathogenesis of mast cell activation by infectious processes remains unclear (13).
Moreover, new episodes of infection are accompanied by reappearance or aggravation of urticaria symptoms, causing chronic spontaneous urticaria (3, 14).
In our study, a clear etiology of AU has not been identified in 32.4% of children (idiopathic urticaria), in agreement with literature data (15, 16). AU secondary to food was found in 14% of the patients. The predominant foods that cause urticaria are milk, eggs, peanuts, tree nuts, fish, and shellfish. Foods were reported to be the possible cause of attacks of AU in 0.9% and 1.3%, respectively, of patients in two previous studies (11, 12); however, Juhlin reported that foods and drinks were associated with exacerbation of wheals in 30% and 18%, respectively, of patients with recurrent attacks of urticaria (17).
Drug treatment used in the management of urticaria was administered in agreement with international guidelines considering the severity of symptoms (*e.g.,* intense pruritus, angioedema) (4, 18). Therefore, we documented that antihistamines were the most common drug used. We did not record any adverse drug reaction, probably because the short duration of treatment.
We found that specific triggers of AU were not associated with severe urticaria, even if we found that unknown causes were significantly associated with mild urticaria and infectious triggers with a moderate one. Also, we found that pre-school children had more frequent mild urticaria ($p < 0.04$). AU has been defined as spontaneous wheals presenting for less than six weeks (1, 19). However, its duration is related to the clinical presentation of disease (15, 20). Therefore, a detailed understanding of the related factors that may influence the duration of AU will help primary physicians to perform a more appropriate clinical assessment. So, we aimed to

Table IV - *Related factors associated with the recurrence of an initial episode acute urticaria in children.*

		RECURRENCE		
		follow-up 1 month	follow-up 3 months	follow-up 6 months
Physician-diagnosed cause of urticaria	Idiopathic	10	11	10
	Infectious	8	6	4
	Food	4	4	2
	Drugs	/	2	/
	Poison	/	/	/
	Contact	4	3	2
Sex	Female	13	13	10
	Male	13	13	8
Age	0-5 years	9	13	8
	6-10 years	9	6	6
	11-15 years	5	5	4
	16-18 years	3	2	0
Severity	Mild	11	9	4
	Moderate	10	11	8
	High	5	6	6
With family allergic history		9	11	10

Figure 1 *- Detailed information on the relationship between etiologies of the first attacks of urticaria and recurrence of urticaria at one, three and six months.*



determine if there were differences in demographic and clinical characteristics in children that had an AU that will progress more probably to CU, with the persistence of symptoms in follow-up at three and six months after the first attack.

We analyzed if there were some patient-related factors associated with persistent urticaria such as age, sex, etiology, severity, and family history. Sex was not a statistically significant factor associated with the duration of urticaria. Children 5-10 years old and 10-15 years old had a greater tendency to CU, with symptoms remaining at six months of 6% and 7%, respectively. About etiology, the higher prevalence of urticaria has been observed in the group with unknown etiology (10%). Severity and familiarity did not seem to correlate with the persistence of urticaria.

The present study has some limitations. Firstly, we have not recorded laboratory data (for example, white blood cell, C-Reactive Protein) because current guidelines do not recommend routine diagnostic tests or extended diagnostic programs in patients with acute urticaria. Moreover, we did not record the total number of accesses of urticaria in pediatric age in Italy, even if the reported data are recorded in several cities of Italy and probably could represent a model of Italian reality.

Conclusions

We believe that the identification of children with AU who have a high risk of progression to CU is essential for better diagnostic and therapeutic management. We think that a good quality standardized questionnaire aimed to identify specific high-risk factors, together with a detailed physical examination, can provide important data related to the progression from AU to CU and guide the follow-up.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Assessment of IgE- and cell-mediated immunity in pediatric patients with eosinophilic esophagitis

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Key words

Eosinophilic esophagitis; skin prick test; prick to prick test; atopic patch test; IgE; non-IgE-mediated food allergy.

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Summary

Eosinophilic esophagitis (EoE) is a chronic allergen/immune-mediated disease leading to esophageal dysfunction. Food allergens play critical roles in the pathogenesis and treatment of EoE via different mechanisms. This study aimed to present the characteristics and evaluate the ability of skin prick test (SPT), skin prick to prick test (SPP) (IgE-mediated), and atopic patch test (APT) (cell-mediated) individually or simultaneously to diagnose food allergy in patients suffering from EoE. This prospective study was conducted on 58 patients with EoE. Seven patients (12.1%) were positive to only one, 3 (5.2%) were simultaneously positive to two, and 32 (55.2%) were simultaneously positive to three tests. Single and double sensitizations were totally 10.4% in IgE-mediated reactions, while 36.5% in cell-mediated reactions. In contrast, poly sensitization (> 2 allergens) was 51.7% in IgE-mediated tests and 20.7% in the cell-mediated test. Multiple sensitization findings showed egg white, milk, yolk, and soy were the most frequent allergens. Our findings indicate that EoE is early onset and associated with multiple food sensitizations, particularly via IgE-mediated mechanisms. These immune-mediated responses encompass both IgE-mediated (SPT and SPP) and cell-mediated (APT) reactions simultaneously not individually. Therefore, employing multiple assays may strengthen the diagnosis of food sensitization.

Introduction

Eosinophilic esophagitis (EoE) is a chronic allergen/immune-mediated disease leading to esophageal dysfunction and is characterized by eosinophil infiltration at least 15 eosinophils per high-power field (HPF), no clinical response to high-dose proton pump inhibitors, and normal pH of the distal esophagus (1). The incidence of EoE has been increased over the past two decades (2). Nevertheless, the role of allergy-mediated responses, clinical manifestation, and the age of onset of EoE is not fully determined. Foods have always been one of the main culprits in the pathogenesis of the disease (3, 4). Dietary therapeutic approaches, including amino acid-based elemental diets, either allergy test-directed elimination diets or non-directed empiric elimination diets resulted in partially clinical and histologic remission and can be implemented as the first-line non-pharmacologic therapies (5, 6). Furthermore, the role of cellular immunity in EoE is attributed to Th2 responses; thus, allergic patch

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test (APT) may be used for type IV cell-mediated immunity (7, 8). Although trial six or ten-food elimination diet was initially suggested, it is difficult to be continued because patients are deprived of major nutritional elements (9). Therefore, finding a non-invasive and accurate test sounds necessary to diagnose food trigger(s) in EoE. Various tests, such as SPT and APT, showed different results in children and adult in detecting food allergens (10). Therefore, this study aimed to assess the most relevant clinical information such as the onset age of symptoms and clinical presentations along with SPT, SPP, and APT either individually or simultaneously to diagnose food allergy in patients with EoE.

Materials and methods

This prospective study was conducted on pediatric patients with EoE referred to the Allergy and Pediatric Gastroenterology Outpatient Clinic of three tertiary Hospitals (including Rasoul Akram, Ali Asghar, and Firooz Abadi Hospitals, Tehran, Iran) from September 2013 to January 2018. The EoE disease was confirmed based on 2011 consensus document (11). Written informed consent was obtained from all participants and the study protocol was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC1396.8923496039). The SPT and SPP tests were used for IgE-mediated reactions, while APT was used for cell-mediated reactions (12-14) Statistical analyses were performed using SPSS version 23.0 (Chicago, Illinois, USA). A p-value of less than 0.05 was considered statistically significant.

Results

Demographic data and clinical manifestation are shown in **table I**. Of the 58 patients with EOE, 37 (64%) were males and 21(36%) were females, the male/female ratio was 1.8:1. The median (IQR) gap between symptom onset and the definite diagnosis was 15 (9.37-21.4) months. The five most common clinical manifestations consisted of anorexia (77.6%), vomiting (69%), abdominal pain (63.8%), nausea (56.9%), and sleep disorder (37.9%). The mean age ± SD at the first endoscopy was 24.4 ± 12.2 months (11 to 60 months). Thirty-one (53.4%) patients were diagnosed at the first endoscopy, 21 (36.2%) at the second time, 5 patients (8.6%) at the third time, and one patient (1.7%) after the fourth endoscopy.

Sixteen patients were negative to all of the skin tests and 42 were reactive to at least one of the allergens in three types of the skin tests. Seven patients (12.1%) were positive to only one kind of skin test, 3 (5.2%) patients were simultaneously positive to two skin tests, and 32 (55.2%) patients were simultaneously positive to the three skin tests. The number of patients with multiple sensitizations (reactive to more than two allergens) in IgE-mediated reactions was 30 (51.7%) cases, whereas in cell-mediated reactions was 12 (20.7%) cases (**table II**). The most frequent food allergen detected by IgE-mediated tests (SPT and SPP) was cow's milk protein (46.6%) and the most frequent detected by APT was egg white (34.5%). Moreover, the SPT and SPP results revealed that the cow's milk, egg white and yolk (more than ten cases), and soy were the most frequent allergens, while APT indicated egg white, cow's milk, and soy. According to the multiple sensitization results, egg white, milk, yolk, and soy were the most frequent allergens. The lowest positive reactions to food allergens in IgE- and cell-mediated reactions were observed for almond (**table III**). Twelve (20.7%) patients were sensitized to yolk in IgE-mediated reactions, but there are only 3 (5.2%) cases in cell-mediated reactions. Multiple sensitizations were more frequent in comparison to single or double sensitizations. Moreover, there was a significant positive correlation between total IgE and both peripheral eosinophil count/mm^3 ($p = 0.03$, $r = 0.28$) and biopsy eosinophil count/HPF ($p < 0.001$, $r = 0.53$). However, no significant correlation was found between biopsy and peripheral eosinophil counts ($p = 0.53$).

Discussion

Skin prick test (SPT or SPP), serum specific IgE assay and APT are the most available allergy tests; however, the specificity and sensitivity of these tests are under investigation (15-17). In parallel with the review of these tests, what is highlighted in our study is the nature of multiple sensitization in this disease, and interestingly, this finding is more evident in the IgE-dependent mechanism than in the cell-dependent mechanism, which 51.7% of the skin tests in IgE-mediated reactions are multiple sensitizations (more than two allergens), while 20.7% of the skin tests in the cell-mediated reactions are multiple sensitizations. On the other hand, there were three cases with single sensitization (5.2%) in IgE-mediated reactions, whereas single sensitization constituted 19% of cell-mediated reactions. Nevertheless, few studies have been done in this area of research. In the present study, the cow's milk, egg white, yolk, and soy were the most frequent food allergens in IgE-mediate skin tests. In contrast, egg white, cow's milk, rice, and soy were the most frequent food allergens in cell-mediated reactions. Given the frequency of multiple sensitizations, egg, milk, and soy were the most prevalent allergens. Mono and multiple sensitizations in allergy tests are topics that we focused on in the current study, otherwise many studies have performed different allergy tests regardless of this issue; for example, Spergel *et al.* (7) showed nearly 70% of the patients were reactive to only 1 to 3 foods. The number of food allergies was consistent with Kagalwalla *et al.* cohort study that 72% of the population was only allergic to one food. Also, they reported that milk, egg, wheat, and soy were the most common food allergens (18). In agreement with the above-mentioned studies, the current study demonstrated that milk, egg, and soy were the most prevalent food allergen.

Table I *- Demographic characteristics and clinical data of the patients with EOE.*

Parameters	EOE patients (n = 58)	
Age of diagnosis (month)	28 (20-36)	
Age of onset (month)	9 (7-14)	
Gender	Female	21 (36%)
	Male	37 (64%) **
Delivery	NVD	25 (43%)
	CS	33 (57%)
Nutrition	breast milk	21 (36.2%)
	Formula	10 (17.2%)
	both	27 (46.6%)**
Age of supplementation food	Before 4 month	3 (5.2%)
	Ranging 4 to 6 month	20 (34.5%)
	End of 6 month	35 (60.3%)***
Allergic family history	Yes	43 (74%)***
	No	15 (26%)
Asthma history	Yes	33 (56.9%)
Allergic Rhinitis history	Yes	36 (62.1%)
Eczema history	Yes	20 (34.5%)
Anaphylaxis history	Yes	11 (19%)
Peripheral eosinophil count/mm^3	395 (188-832)	
Serum IgE (IU/mL)	170 (70-332.5)	
Tissue Eosinophil count /HPF	26(19-32)	

Cesarean Section (CS).
Normal Vaginal Delivery (NVD).
Data were presented as median (IQR) and frequency (%).
Using one-sample chi-square test the p-value < 0.01 and < 0.001 presented as ** and ***, respectively.

Table II *- The results range from single sensitization to multiple sensitizations.*

Skin test	Single sensitization	Double sensitizations	Multiple sensitizations	Totally: Negative vs. Positive
IgE-mediated (%)	3 (5.2)	3 (5.2)	30 (51.7)***	22 (37.9) 36 (62.1)**
Cell-Mediated (%)	11 (19)	9 (15.5)	12 (20.7)	26 (44.8) 32 (55.2)
IgE- and cell-mediated (%)	7 (12.1)	3 (5.2)	32 (55.2)***	16 (27.6) 42 (72.4)**

The p-value<0.01 and <0.001 presented as ** and ***, respectively.

Table III - *The frequency of allergens based on types of skin tests.*

Allergens	**Skin Tests**						
	IgE-mediated		Cell-mediated	^^SPT + SPP + APT			
	^SPT	^SPP	^APT				
	Positive (%)	**Positive (%)**	**Positive (%)**	**Negative (%)**	**Positive (%) †**		
					Single Allergen	**Double Allergens††**	**Multiple Allergens†††**
Milk	22 (37.9)	27 (46.6)	13 (22.4)	29 (50)	1 (1.7)	2 (3.4)	26 (44.1)**
Egg white	19 (32.8)	23 (39.7)	20 (34.5)	28 (48.3)	1 (1.7)	2 (3.4)	28 (47.5)**
Yolk	12 (20.7)	12 (20.7)	3 (5.2)	38 (65.6)	1 (1.7)	1 (1.7)	18 (30.5)**
Soya	11 (19)	12 (20.7)	8 (13.8)	40 (70)	2 (3.4)	1 (1.7)	16 (27.6)**
Wheat	8 (13.8)	3 (5.2)	4 (6.9)	50 (86.2)	1 (1.7)	0	7 (12.1)
Meat	6 (10.3)	3 (5.2)	2 (3.4)	51 (87.9)	1 (1.7)	0	7 (12.1)
Peanut	6 (10.3)	5 (8.6)	5 (8.6)	45 (77.6)	1 (1.7)	1 (1.7)	10 (17.2)**
Fish	4 (6.9)	1 (1.7)	1 (1.7)	53 (91.4)	0	0	5 (8.6)
Rice	4 (6.9)	4 (6.9)	9 (15.5)	47 (81)	0	0	11 (19)
Chicken	3 (5.2)	1 (1.7)	1 (1.7)	55 (94.8)	0	0	3 (5.2)
Sesame	3 (5.2)	5 (8.6)	5 (8.6)	49 (84.5)	0	0	9 (15.5)
Almond	2 (3.4)	1 (1.7)	0 (0)	56 (96.6)	0	0	2 (3.4)

^ Each test is assessed alone. ^^ tests are assessed simultaneously.
† The positive results for three skin tests were considered totally for different food allergens.
†† Double allergens were considered when that allergen was positive with another food at the same time in one kind of tests.
††† Multiple Allergens were considered when that allergen was positive with more than 2 foods at the same time in one kind of test.The p-value<0.01 and <0.001 presented as ** and ***, respectively.

Finding the exact food allergen as a true trigger is the main challenging topic in these dietary therapies. Most studies have shown that the use of different methods can increase the chance of success in identifying allergens. However, there are some problems regarding this attitude; for example, basophil activation test is not available everywhere and APT is not well-standardized for implementation or interpretation (19, 20). Consistent with these studies, Philpott *et al*. declared that SPT, APT, sIgE, and BAT could not predict the exact food sensitivity related to EoE in comparison to oral food challenge as a gold standard test. We demonstrated that multiple assays can simultaneously increase the chance of finding positive sensitization results and may help find true trigger(s) to manage appropriate dietary therapy. But there are still two major challenges: first, these findings can only represent sensitization, not the main culprit and second, several studies previously established that local specific IgE production may play an important role in the pathogenesis of EoE (21, 22) and these tests miss the local IgE production. Gottlieb *et al*. (23) concluded that directed dietary elimination and reintroduction based on SPT/APT is not a worthwhile treatment approach, while other studies (24, 25) suggested that diet modification based on SPT/APT is a valuable approach in patients with EoE. On the other hand, the most limitation of the test-guided approach is its inability to detect true allergens. We discussed that this study along with other available evidence suggests that the combination of different tests may increase the success rate for food allergen detection and another important finding is the nature of polysensitization in the pathogenesis of EoE, especially in IgE-mediated mechanism.

Our findings revealed that EOE is early onset and associated with multiple sensitizations to common food allergens. Although both IgE- and cell-mediated mechanisms are suggested in the pathogenesis, it appears that their impact and role in the pathogenesis are different and they may be helpful to categorize patients into IgE- or cell-mediated groups; accordingly, therapeutic approaches may be different. The mixed IgE- and cell-mediated nature of

this disease necessitates the use of different laboratory methods that may elucidate different mechanisms.

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Conflict of interests

The authors declare they have no conflict of interests.

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Omalizumab is effective in patients with chronic spontaneous urticaria plus multiple chronic inducible urticaria

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To the Editor,

Chronic urticaria (CU) is characterized by wheals and/or angioedema for more than 6 weeks, with a point prevalence of 0.1-1.4% (1, 2). CU is classified as chronic spontaneous urticaria (CSU) and chronic inducible urticaria (CIndU), which can occur simultaneously or independently (3). In CSU patients, concomitant CIndU has been associated with severe, long-lasting and/or antihistamine-resistant CSU (4). Omalizumab, an anti-IgE monoclonal antibody, can improve both CSU and CIndU in the same patients (5, 6). However, its efficacy in CSU patients with several subtypes of CIndU is poorly characterized (6, 7). Here, we describe six patients with antihistamine-resistant CSU and multiple CIndUs (**table I**) treated with 300 mg omalizumab monthly and followed up for a period of 3-11 months.

Common blood count and serum levels of total IgE, C-reactive protein (CRP), eosinophil cationic protein (ECP), D-dimer and fibrinogen were measured at baseline and 30 days after the injection of omalizumab. The Urticaria Activity Score (UAS) was obtained for 30 days (before and during the treatment). Dermatology Life Quality Index (DLQI) was used at baseline and every 7 days. The Chronic Urticaria Quality of Life Questionnaire (CU-Q2oL) and the Urticaria Control Test (UCT) were applied at baseline and 30 days after the first omalizumab injection. Provocation tests with appropriate triggers were performed according to the international guideline (1). The age of patients ranged from 26 to 52 years (mean: 43 years, **table I**). The mean duration of CSU and CIndU was two and three years, respectively. In three cases, CIndU appeared before CSU, and in the other three patients CIndU developed after or at the same time as CSU. Provocation tests were positive before treatment in all

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Table I *- Demographic characteristics of patients.*

#	Sex	Age, years	Duration of CSU/ CIndU, years	Type of CIndU	Before/4 weeks after the first omalizumab injection							Response to omalizumab (UAS7), after 1st/2nd injection**	Speed of response***
					Presence of angioedema	UCT	DLQI	CU-Q2oL, %	Provocation tests*	Total IgE, IU/ml (< 114)	D-dimer, ng/ml (< 500)		
1	F	27	3/3	SD, DPU, LHU	No/No	11/12	15/4	51/70	All CIndUs +/-	50/195	86/98	Partial/ Complete	Fast
2	F	26	6/5	ChU, ColU, SD	Yes/No	7/13	14/11	48/60	All CIndUs +/-	51/190	297/285	Partial/ Complete	Fast
3	F	31	1/2	ChU, SD, DPU	No/No	10/12	13/8	54/66	All CIndUs +/-	8/54	206/212	Partial/ Complete****	Fast
4	M	26	1/1	SD, DPU	No/No	11/15	12/8	52/69	SD +/- DPU +/+	15/87	204/239	Partial/ Complete****	Slow
5	F	45	2/3	ChU, SD, DPU	Yes/No	9/12	16/9	46/61	All CIndUs +/-	30/90	563/582	Partial/ Complete****	Fast
6	F	52	3/4	ChU, SD	Yes/No	9/12	14/6	58/79	ChU +/- SD +/+	96/183	652/645	Partial/ Partial****	Slow

UCT: Urticaria Control Test; DLQI: Dermatology Quality of Life Index; CU-Q2oL: Chronic Urticaria Quality of Life Questionnaire; CIndU: chronic inducible urticaria; F: female; M: male; SD: symptomatic dermographism; DPU: delayed pressure urticaria; LHU: local heat urticaria; ChU: cholinergic urticaria; ColU: cold urticaria; *results of provocation tests: "+" is positive provocation test and "-" is negative provocation test; patients #4 and #6 had slightly positive provocation tests for DPU and SD, respectively, after omalizumab treatment; **complete response: >90% reduction from baseline in UAS7 score and partial imrovement: 30-89% reduction from baseline in UAS7 score; ***fast responder: CSU symptoms regressed within 8 days and slow responder: CSU symptoms regressed after 8 days; ****response after the second injection was determined by physician global assessment based on patient feedback.

patients. Concomitant symptomatic dermographism (SD), delayed pressure urticaria (DPU) and cholinergic urticaria were diagnosed in six, four, and four patients, respectively. Two and four CSU patients had two and three different subtypes of CIndU, respectively. All patients had wheals and three of them experienced several episodes of angioedema. All patients had uncontrolled disease.

In all patients, disease control was reached, and quality of life was improved within one month after the first omalizumab injection (**table I**). Provocation tests became negative in four patients. In two patients with SD or DPU, provocation tests remained slightly positive, but everyday CIndU symptoms were gone.

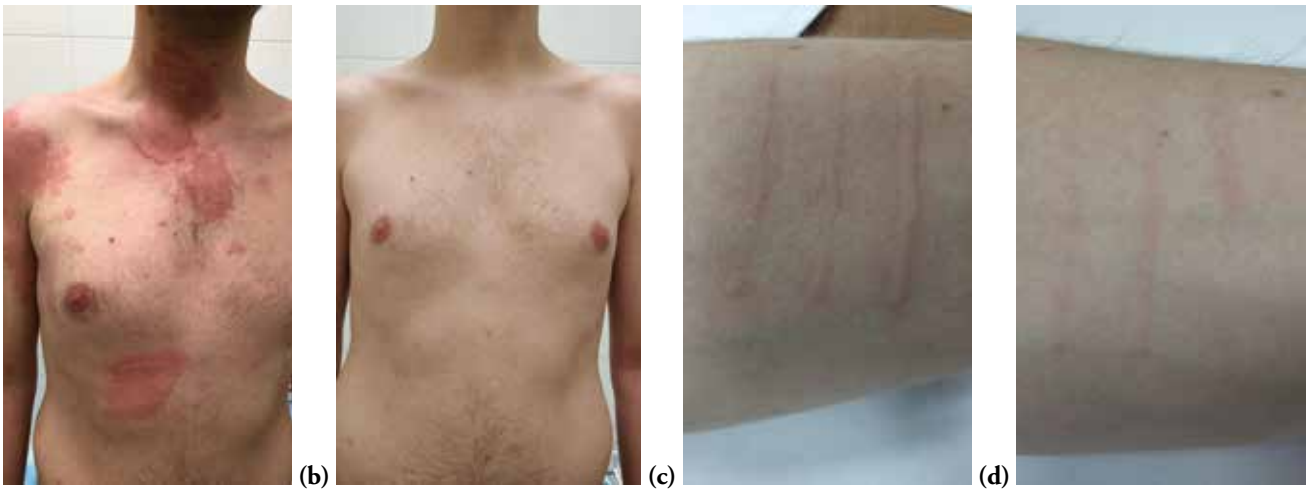
Partial improvement assessed by UAS7 was seen in all patients after the first injection and five of six patients reported complete remission of their CSU after the second injection. Four patients were fast responders and two were slow responders as assessed by UAS and UCT scores (**table I**). In five patients, the symptoms of CSU and CIndU decreased at the same time. In two slow responders, CIndU symptoms disappeared 2-3 weeks before improvement of CSU symptoms (**figure 1 a-d**).

In all patients, total IgE levels were elevated after the treatment as compared with their baseline values (**table I**) as described before (8). In the literature, low levels of total IgE at the baseline have been reported to be associated with nonresponse and/or slow response to omalizumab (9). However, we did not observe this in our patients probably because of the small number of patients included.

Similarly, decrease in CRP and D-dimer levels after successful treatment with omalizumab has been reported (8). In our patients, no difference was seen in levels of ECP, D-dimer, CRP and fibrinogen before and after treatment. In two patients, elevated D-dimer levels were present before the onset of their urticaria and might be associated with concomitant diseases.

The pathomechanism of chronic urticaria is yet to be clearly defined. It is still unknown what causes this activation and degranulation of tissue-resident mast cells and the subsequent release of inflammatory mediators. Type I autoimmunity ("autoallergy") is thought to be a cause of both CIndU and CSU in a subpopulation of patients. Autoallergic urticaria is characterized by the synthesis of autoantigen (autoallergen), which is detected by specific IgE autoantibodies bound to skin mast cells that resuts in degranulation of mast cells. For example, some IgE autoantibodies have been described in CSU, namely IgE against thyroid peroxidase, interleukin-24 and tissue factor. In patients with Type I urticaria, omalizumab can prevent binding of IgE to the high-affinity IgE receptor and, therefore, suppress mast cell activation and release of histamine and other mediators. However, some CSU patients respond more slowly to omalizumab that is consistent with Type IIb autoimmunity associated with IgG autoantibodies against IgE and FcεRI. In these patients, treatment

Figure 1 *- Patient #4. CSU: before* ***(a)*** *and after* ***(b)*** *omalizumab treatment. Symptomatic dermographism (results of provocation test with FricTest® device): before* ***(c)*** *and after* ***(d)*** *omalizumab treatment.*



with omalizumab can result in the loss of membrane-bound IgE and subsequently FcεRI from skin mast cells that prevents the activation of mast cells by IgG autoantibodies (1, 5, 10, 11). In line with other publications (5, 6, 10-12), our patients with CSU plus several subtypes of CIndU responded well to omalizumab treatment, which resulted in decreased urticaria activity, provocation test responses and increased quality of life and disease control. Prospective treatment studies with omalizumab, in patients with CIndU with and without CSU, children and adults, should be performed.

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Conflict of interests

Pavel Kolkhir is a speaker for Novartis and Roche. Anastasiia Allenova is a speaker for Novartis.
Dayana Skander has no conflict of interest. Marcus Maurer: grants and/or personal fees from Allakos, Aralez, Alnylam, AstraZeneca, BioCryst, Blueprint, CSL Behring, FAES, Genentech, Kalvista, Menarini, Leo Pharma, Moxie, MSD, Pharming, Pharvaris, Roche, Sanofi, Shire/Takeda, UCB, Uriach, outside the submitted work.

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Hymenoptera Venom Allergy: Re-Sting reactions

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Key words

Hymenoptera venom immunotherapy; re-sting reactions; severity; effective; Hymenoptera Venom Allergy

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To the Editor,

Hymenoptera Venom Allergy (HVA) is a serious and potentially fatal disease, and it is one of the major causes of anaphylaxis (1). The prevalence of hymenoptera stings in the general population ranges from 56.6% to 94.5%, and it may vary according to the location and the climatic conditions whereas HVA (2) affects up to 5% of the general population and up to 32% of beekeepers (3). Clinical studies reveal certain predispositions that may affect the severity of the allergic reactions to Hymenoptera venom, for example, age, cardiovascular diseases, drugs, in particular beta-blockers and Angiotensin-Converting Enzyme inhibitors (ACEi), the number of stings per year and atopic diseases (4).
Due to the fear of future reactions, HVA imposes a significant impact in health-related Quality of Life (QoL) (5). Hymenoptera Venom Immunotherapy (VIT) is the only effective treatment in HVA. It is safe and it induces tolerance to hymenoptera venom, providing long-term protection from further systemic reactions in 95% of patients allergic to wasp venom, and approximately 80% of those allergic to bee venom (3).
VIT consists in the subcutaneous administration of the selected venom extract with an initial induction phase, followed by a maintenance phase that usually consists in the administration of 100 µg of venom extract at scheduled intervals. The overall duration of VIT is 3 to 5 years but it may be longer in selected patients (5-7). VIT reduces the risk of subsequent systemic sting reactions to as low as 5% compared with the risk of such reactions in untreated patients for whom the risk may be as high as 60% (2). The overall relapse rate after discontinuation of VIT is 10%-15%. The risk is higher in patients who were treated for less than 5 years (8).
The aim of this study was to analyze hymenoptera re-sting reactions in patients with indication for VIT. A secondary objective was to evaluate differences in the severity of reactions of re-stings between patients who underwent VIT or not.

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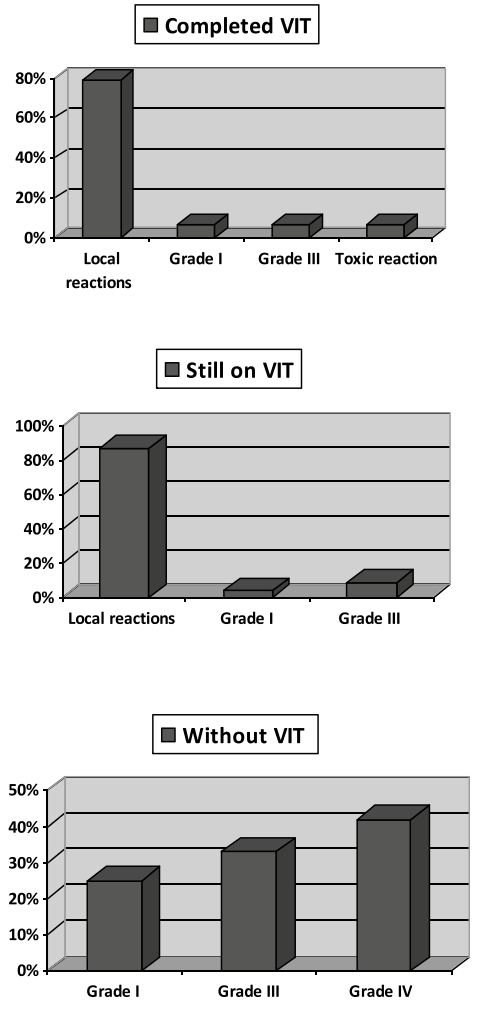
Figure 1 *- The severity of the reactions, according to Mueller's grade, of the patients who were re-stung after completing VIT, during VIT and did not undergo VIT.*



A medical records review of all patients with indication for VIT according to the European Academy of Allergy and Clinical Immunology (EAACI) guidelines (9) between 2005 and 2016 in our Clinical Allergy Department was performed.

Data regarding demographics, sting reaction severity according to Mueller's criteria (10), hymenoptera involved, specific IgE, venom skin tests, date of proposal, first and last administration and treatment completion was collected.

A structured questionnaire was applied by telephone to the patients.

Statistical analysis was performed using IBM SPSS Statistics®, version 24.0. Continuous variables are expressed as means and standard deviations; categorical variables were expressed as frequencies and percentages. A p value < 0.05 was considered statistically significant.

A total of 113 patients were included: 80 (71%) males, with a mean age of 38 (± 15) years. Of these, 23 (21%) were beekeepers and 25 (23%) were atopic. With respect to atopic diseases, 4 (4%) had asthma and 14 (13%) rhinitis. Other comorbidities included cardiovascular disease in 18 (16%) and 14 of these patients were on ACEi and/or beta-blockers.

VIT with honeybee was proposed in 73 (64%), wasp 38 (34%) and *Polistes* 2 (2%). The mean duration of VIT was 45 (± 16) months. However, 23 completed less than 36 months of treatment.

Unfortunately, 28 patients (25%) were not treated with VIT. The main reason admitted for not complying with the treatment was economical (3). VIT was entirely supported by the patients at that time without any type of reimbursement.

Eighty-eight patients (78%) participated in the telephone interview. Of these, 49 (56%) completed VIT, 15 were still on VIT (17%) and 24 (27%) did not undergo treatment. Of those who completed VIT, 14 (29%) were re-stung and 3 went to the Emergency Department (ED). Twenty-four patients (38%) were stung while still on VIT. Twelve (50%) of those who did not perform VIT were re-stung and 9 went to the ED.

The severity of the reactions, according to Mueller's grade (10), of the patients who were re-stung after completing VIT was: local reactions in 11 (79%), grade I in 1 (7%); grade III in 1 (7%). One had a toxic reaction after multiple stings. The mean follow-up time was 45 (1-110) months. Those who were stung during VIT, 20 (87%) had local reactions, 1 (4%) grade I and 2 (9%) grade III. Of those who did not undergo VIT and were re-stung: 3 (25%) had grade I, 4 (33%) grade III and 5 (42%) grade IV (**figure 1**).

In this series, the patients who were not treated with VIT had a greater number of systemic reactions when re-stung as well as more severe reactions ($p < 0.01$).

In this group, re-sting reactions were less severe in the patients who had completed or who were on venom immunotherapy, as expected. Three quarters of those who did not undergo treatment had severe anaphylactic reactions when re-sting.

VIT is recommended in individuals (children and adults) with documented sensitization to the venom of the culprit insect and systemic sting reactions exceeding generalized skin symptoms (such as pruritus, flushing, urticarial and angioedema) and adult patients with generalized skin symptoms if quality of life is impaired (9).

This study reinforces that venom immunotherapy is highly effective in the treatment of hymenoptera venom allergy and it should be widely available for all patients with HVA at risk.

Conflict of interests

The authors declare that they have no conflict of interests.

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