edra | Issn 1764-1489 | Volume 46 N. 6/2014 – November 2014

European Annals of Allergy and Clinical Immunology

THE OFFICIAL JOURNAL OF AAITO | ASSOCIAZIONE ITALIANA ALLERGOLOGI IMMUNOLOGI TERRITORIALI E OSPEDALIERI
THE OFFICIAL JOURNAL OF SPAIC | SOCIEDADE PORTUGUESA DE ALERGOLOGIA E IMUNOLOGIA CLINICA

6/2014

European Annals of Allergy and Clinical Immunology

THE OFFICIAL JOURNAL OF AAITO
ASSOCIAZIONE ITALIANA ALLERGOLOGI IMMUNOLOGI TERRITORIALI E OSPEDALIERI

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

edra

Editors in Chief
Riccardo Asero
Mário Morais-Almeida

Publishing Director
Nicola Miglino

Publishing Editor
Chiara Scelsi
c.scelsi@lswr.it
Tel. 02 88184.257

Production Manager
Walter Castiglione
w.castiglione@lswr.it
Tel. 02 88184.222

Sales & Marketing
Ludovico Baldessin
l.baldessin@lswr.it
Tel. 02 88184.354

Traffic
Donatella Tardini
d.tardini@lswr.it
Tel. 02 88184.292

Subscription
abbonamenti@lswr.it - Tel. 02 88184.317 - Fax 02 88184.151
Italy subscription: 60 euro
World subscription: 85 euro
www.eurannallergyimm.com

Printing
ProntoStampa Srl
Via Praga, 1 - 24040 Verdellino (BG)

EDRA LSWR SpA
Via G. Spadolini, 7
20141 Milano - Italy
Tel. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
www.edizioniedra.it

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

The contents of this Journal are indexed in PubMed and SCOPUS®

Author Guidelines

European Annals of Allergy and Clinical Immunology will accept for publication suitable manuscripts dealing with the aetiology, diagnosis, and treatment of allergic and immunologic diseases. These might include the study of methods of controlling immunologic and allergic reactions, human and animal models of hypersensitivity and other aspects of basic and applied clinical allergy in its broadest sense.We encourage case reports that focus on topic(s) of extreme contemporary interest. Paper reporting the results of drug trials will be considered.
European Annals of Allergy and Clinical Immunology also publishes solicited and usolicited review articles on subjects of topical interest to clinical and experimental allergy.

Manuscript

We request that all manuscripts should be submitted online through our web-based peer review system.
Submitted contributions are accepted for publication on the basis of scientific interest and relevance, at the final discretion of the Editors in Chief, who will have blinded written evaluations from at least two anonymous reviewers.
Once a manuscript has been accepted for publication, Authors will receive an electronic page proof for review and approval, following which the manuscript is published in the print journal and on the journal website.
Following acceptance, Authors are also requested to return both completed and signed Journal Publishing Agreement and Conflict of interest disclosure forms by e-mail to: c.scelsi@lswr.it

Full Authors Guidelines, online Submission System link, Journal Publishing Agreement and Conflict of interest forms are available on Journal website: www.eurannallergyimm.com
Typed manuscripts at 30 lines per page: maximum lenght 10 pages, around 300 lines.
Manuscripts should be typewritten (double spacing) on one side of the paper; on a separate sheet, should bear the title of the paper, name, postal and e-mail address of the Author, together with the name of institution where the work was done.
Generally, papers should be divided into the following parts and in the order indicated:

1. **Summary and key words**: english, limited to 15 lines.
2. **Introduction**: containing the reasons for doing the work.
3. **Materials and methods**.
4. **Results**: these should be given concisely; the use of tables and figures to illustrate the same results will only rarely be allowed.
5. **Discussion**: the presentation of results should be separated from a discussion of their significance.
6. **References**.

Units and Abbreviations

European Annals of Allergy and Clinical Immunology recognizes the adoption of the International Systems of Units (SI-Units). Abbreviations to be put in a glossary at the foot of page 1 on the text.

References

References should be in the order:

- the order number corresponding with that of appearance in the text;
- the author's name(s), followed by initial or first name;
- the title of the work, in the original language;
- for journals: usual title abbreviations according to international nomenclature and in the order: year, volume number, issue number (in parenthesis), first and last page numbers of the work.

For example:
Bodtger U, Linnegerg A. Remission of allergic rhinitis: An 8-year observational study. J Allergy Clin Immunol 2004; 114(6): 1384-8.

- for books: name of the author/editor, title, publisher/institution, town where published, year of publication, first and last page numbers of the work.

For example:
Paupe J, Scheinman P (Eds.). Allergologie Pédiatrique. Flammarion, Paris, 1988: 324-42.

Illustrations

- Figures always on separate numbered sheets and legends on the back in pencil
- Figures always saved on separate numbered files
- Figures, diagrams: JPG, 300 dpi minimum
- Radiographs: JPG, 300 dpi minimum

All tables, figures, radiographs, etc. must be referenced in the text.
Legends should be put on a separate sheet, saved on a separate file and have the same numbers as the figures.

The "pdf " of the article will be sent to the author by e-mail.

EDRA LSWR SpA
Via Spadolini, 7
20141 Milano - Italy
Tel. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
www.eurannallergyimm.com

Table of Contents

REVIEW | Eur Ann Allergy Clin Immunol | Vol 46, N 6, 196-200, 2014

E.R. Madonini

Probiotics and allergies: myth or reality?

Allergopharma S.p.A. Scientific Direction

Key words
Probiotics; immune system; allergic diseases; atopic dermatitis; allergic rhinitis

Corresponding Author
Enzo R. Madonini
Via C. Porta-Residenza Bosco 833
20080 Basiglio (MI)
Phone: +39 335 52 62 740
E-mail: enzo.madonini@gmail.com
enzo.madonini@external.allergopharma.com

Summary
During the last years, along with the growing knowledge about the role and importance of the intestinal flora, interest remarkably increased in probiotic bacteria supplementation. It has indeed been demonstrated that the intestinal microbiota is very important in the regulation of several functions of the organism, even those far from the gastro-enteric system. Among them, great interest was stimulated by the proven capability of the intestinal microbiota to regulate the immune system, in particular to rebalance the TH1/Th2 ratio. Consequence thereof is the assumption that the administration of probiotic bacteria may induce clinical benefits in allergic pathologies. Many clinical studies have been carried out that considered the possibility of preventing allergic sensitizations, and preventing and treating atopic dermatitis and allergic rhinitis. Many studies demonstrated that the administration of probiotics is able to prevent the onset of allergic sensitizations and improve the symptoms of atopic dermatitis and allergic rhinitis; however, studies were published, too, that achieved negative outcomes. The overall evaluation of results is, however, difficult, as the strains used and the study design are markedly heterogeneous. Future investigations with a better standardization will be able to better explain the role of the intestinal flora in atopy, and the role of probiotics in the treatment of allergic diseases.

Introduction

In the early 20th century, the Russian scientist Il'ja Il'ič Mečnikov, who was to be awarded with the Nobel prize for his studies about phagocytosis, first assumed that the particular longevity of some Eastern European populations was due to the wide consumption of acid milk: thus, he called *Lactobacillus bulgaricus* the bacillus he identified as responsible for the process. However, only in 1965, the term "probiotics" was mentioned in an article published in "Science" (1). At present, the interest in the use of probiotics is very high, as shown from the dizzily increasing number of publications in Medline during the last 10 years, paralleling the investigations about the role and importance of the intestinal bacterial flora in regulating numerous functions, even in organisms and systems far from the gastrointestinal tract. Indeed, probiotics:

- promote the exclusion of antigens by increasing degradation and altering their immunogenicity, thus reducing the antigen load;
- regulate the secretion of pro- and anti-inflammatory mediators, orienting the development of the immune system;
- bring the properties of an unbalanced microbiota back to normal;
- normalize increased gut permeability, that is the outcome of the inflammation of the mucosa;
- inhibit the colonization by pathogenic microorganisms via the production of anti-microbial substances (post-biotics), and/or through competition by adhesion to the mucosa (2,3).

Among these functions, the ability of the intestinal flora to modulate the activity of the immune system seems particularly interesting. The mechanisms most frequently observed are the following:

- immunomodulation (induction of T_{reg} cells, re-alignment of the Th1/Th2 ratio);
- secretion of Th1 cytokines (IL-10, IL-12, TGF-b);
- decreased IgE production;
- development of tolerogenic dendritic cells;
- activation of NK cells;
- stimulation of Toll-like receptors;
- production of secretory IgA (2,4-6).

In particular, the modulation of the Th1/Th2 ratio together with the increased number of T_{reg} cells and their functions allowed assuming the clinical efficacy of probiotics in the field of allergies.

Probiotics and allergies

Some preliminary observations are particularly interesting. First, germ-free animals are characterized by important immunologic anomalies:

- less developed Peyer plaques and mesenteric lymph nodes;
- smaller number of B and T cells;
- absence of germinal centres;
- smaller number of DC, $CD4^+$ T cells, IgA-specific B cells;
- small-sized spleen;
- T_h2-type immune responses;
- reduced $CD4^+CD25^+$ number and activity;
- increased inflammatory response to provocation tests.

All the above-mentioned alterations are reversible with probiotics supplementation (7). The observations about the intestinal flora of atopic children are particularly important. Epidemiologic data have shown that the bacterial flora in these children is different from that of healthy children, with higher levels of *clostridia* and lower levels of *bifidobacteria*. Other trials pointed out that an early colonization with potentially pathogen bacteria, such as Clostridium difficile and Staphylococcus aureus, mainly occurs in children who will develop allergy. On the contrary, *lactobacilli* and *bifidobacteria* are most commonly present in the intestinal flora of healthy children (8-11). Hence, the usefulness of submitting these patients to daily long-term probiotics supplementation was assumed, in order to modulate the immune system in the anti-atopic sense.

Clinical studies about probiotics and allergies

The first DBPC trial about the use of probiotics in allergies dates back to 2001, only (12). Since then, most studies on probiotics were performed mainly in the following three allergy situations:

1. prevention of the onset of allergic sensitization;
2. prevention and treatment of atopic dermatitis;
3. prevention and treatment of allergic rhinitis.

Prevention of the onset of allergic sensitization

An important meta-analysis has recently been published on this topic: it considered 25 studies and as many as 4031 patients (13). This study observed a significant IgE decrease as well as allergic sensitization in treated subjects after the administration after and before birth, i.e. to pregnant women. Outcomes about clinical symptoms were conflictual. However, some Authors do not share this observation.

Prevention and treatment of atopic dermatitis

As for this pathology, contrasting outcomes have been observed as for the possibility of *preventing* its onset: the most favorable evidence was observed in IgE sensitized children. Several studies show that the major benefits are achieved when probiotics are administered *before* and *after* birth. The long-term persistence (5-7 years) of prevention effects was further observed, together with the significant decrease of positive results at prick-tests for ubiquitous allergens. Better results were observed in some studies about the treatment of atopic dermatitis, as for both symptoms and SCORAD levels (14-17). Moreover, it is interesting to observe that a recent wide review of the various nutrient supplements identified in probiotics the most effective substances in the prevention and reduction of atopic dermatitis (18).

Prevention and treatment of allergic rhinitis

Allergic rhinitis seems to be the atopic pathology in which the best clinical results were achieved using probiotics supplementation. Most studies show beneficial effects on seasonal and perennial allergic rhinitis as for symptoms, drug consumption and QoL (15,19,20).
The administration of probiotics showed to alleviate the symptoms of rhinitis and to interfere with inflammation markers, decreasing the eosinophilic inflammation of the mucosa (21) and the production of IL-5 (22). On the contrary, an increased IL-10 and IL-12 production was observed (23). **Table 1** summarizes the characteristics of the main DBPC studies carried out with probiotics in allergic rhinitis. As it can be seen, the pattern of these studies is characterized from marked heterogeneity, as for population, strain used, duration and outcomes. A very recent large study performed by GA_2LEN (24) has to be mentioned: it involved 425 adult patients suffering from grass pollen allergy (215 active, 210 placebo patients) who were treat-

ed for 7 weeks with Lactobacillus paracasei (LP-33®) 2 x 10^9 CFU/die. The study was carried out during the pollen season. It provided for a 10 days' run-in period with Loratadine, followed from the randomization of patients to treatment with LP-33 + Loratadine or Placebo + Loratadine for 5 weeks, and finally a further two weeks' treatment after the discontinuation of antihistamine. Patients treated with the probiotic strain showed a very significant RQOL improvement (mod. from Juniper, JACI 2008) in comparison with baseline and the Placebo group. This wide DBPC study demonstrates that the combination of a probiotic strain and the traditional anti-allergy therapy significantly improves the quality of life of patients suffering from grass pollen-induced allergic rhinitis.

Finally, the possible interactions between probiotics and allergen-specific Immunotherapy (AIT) should be mentioned. The immunomodulatory characteristics of some probiotic strains suggest that they can be used together with AIT to strengthen its effect. Some strains specifically selected because of their ability to induce a marked production of IL-10 and IL-12, such as Lactobacillus plantarum or Bifidobacterium bifidum, were shown to increase tolerance after SLIT in OVA-sensitized mice, at least partially strengthening the Th1 and T_{Reg} response (25-27). No study of this sort was carried out in allergic patients.

Comments

Studies about the use of probiotics in allergic pathologies often achieve contrasting outcomes. It is indeed not a clear field and the interpretation of results is subject to numerous biases. The most relevant are the following:

Table 1 *- DBPC studies of probiotics in allergic rhinitis.*

Author	Year	Strain s	Target	Weeks	Endpoints	Outcomes
Singh	2013	Bif. lactis NCC2818	Seasonal rhinitis	8	Symptoms and cytokines	Improvement
Lin	2013	Lact. salivarius	Perennial rhinitis; children	12	SMS	Reduction
Koyama	2010	Lactobacillus rhamnosus GR-1; Bif. adolescens	Seasonal rhinitis	2 seasons	Symptoms and cytokines	Improvement of cytokines
Chen	2010	Lactobacillus gasseri A5	Pediatric asthma	8	Symptoms, cytokines and PEF	Improvement
Nagata	2010	Lactobacillus plantarum No.14	Seasonal rhinitis	6	Symptoms, cytokines and eosinophils	Improvement
Ouwehand	2009	L. acidophilus; B. lactis	Birch rhinitis	16	Score cytokines	Trend far improvement
Kawase	2009	Lactobacillus GG (LGG) L. gasseri TM C0356	Cypress rhinitis	10	Symptoms and cytokines	Improvement
Xiao	2007	Bif. longum	Cypress rhinitis	4 + 4 cross over EEC	Symptoms	Reduction ocular symptoms
Tamura	2007	Lact. casei Shirota	Cypress rhinitis	8	Symptoms	Poor results
Giovannini	2007	Lactobacillus casei	Asthma and rhinitis; children	54	Free interval from exacerbations	Rhinitis improvement
Ishida	2005	Lactobacillus acidophilus ceppo L-92	Perennial rhinitis	8	Symptoms	Improvement
Helin	2002	Lactobacillus rhamnosus	Birch asthma and rhinitis; children	5,5 months	SMS	negative

- type of probiotic (alive/dead, different strains);
- time of administration (before birth, after birth, before and after birth);
- duration and dosage of administration;
- probiotic mix or other combinations;
- **not comparability of studies carried out using different strains: the results observed with a given strain cannot be extrapolated to other strains, though of the same genre or species;**
- subject-related factors (genetics, atopy);
- environmental factors (microbial load, microbiota, nutrition, pharmacological treatments, country of origin).

In view of these discrepancies, a recent WAO document about the use of probiotics in pediatric allergology stated: "Probiotics do not have an established role in the prevention or treatment of allergy" (28). This is, however, not a failure, as the Authors themselves write in their Take Home Messages: "This is not to say that "probiotic hypothesis" is a dead end. On the contrary, there is tantalizing evidence in vitro and in animal models that the future lies in this direction". After all, the FAO and WHO are recognizing the existence of adequate scientific proofs demonstrating that the consumption of probiotics-containing food may beneficially affect health. They also explain that these effects concern gastro-intestinal infections, some intestinal diseases, urogenital diseases, allergies and infections, they all being disorders affecting large part of the world population (Food and Agriculture Organization and World Health Organization expert consultation: Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Cordoba, Argentina. http://www.fao.org/documents/pub_dett.asp?lang=en&pub_id=61756).

Final Messages

- Intestinal flora plays a fundamental role in several physiopathological processes even far from the gut.
- Numerous evidences show that restoring the activity of the flora through the administration of probiotics is associated with clinical benefits in various pathological situations.
- In particular, several evidences exist that relate intestinal flora with immunity levels and with allergy.
- Furthermore, evidences are available showing that the administration of some probiotic strains interferes with the natural history of allergy and with existing symptoms.
- Evidences and conflicting positions are mainly due to the different characteristics of each individual strain, and to problems linked with the methodology of the studies carried out and evaluated.

Conflict of interest

Dr. Madonini is Scientific Director of Allergopharma S.p.A

References

1. Lilly DM, Stillwell RH. Probiotics: Growth-promoting Factors Produced by Microorganisms. Science. 1965;147(3659):747-8.
2. Klaenhammer TR, Kleerebezem M, Kopp MV, Rescigno M. The impact of probiotics and prebiotics on the immune system. Nature Reviews Immunology. 2012;12(10):728-34.
3. Isolauri E, Rautava S, Salminen S. Probiotics in the development and treatment of allergic disease. Gastroenterology clinics of North America. 2012;41(4):747-62.
4. Kim JY, Kwon JH, Ahn SH, Lee SI, Han YS, Choi YO, et al. Effect of probiotic mix (Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus) in the primary prevention of eczema: a double-blind, randomized, placebo-controlled trial. Pediatr Allergy Immunol. 2010;21(2 Pt 2):e386-93.
5. Dongarra ML, Rizzello V, Muccio L, Fries W, Cascio A, Bonaccorsi I, et al. Mucosal immunology and probiotics. Current allergy and asthma reports. 2013;13(1):19-26.
6. Ozdemir O. Various effects of different probiotic strains in allergic disorders: an update from laboratory and clinical data. Clinical and experimental immunology. 2010;160(3):295-304.
7. Hormannsperger G, Clavel T, Haller D. Gut matters: microbe-host interactions in allergic diseases. J Allergy Clin Immunol. 2012;129(6):1452-9.
8. Sjogren YM, Jenmalm MC, Bottcher MF, Bjorksten B, Sverremark-Ekstrom E. Altered early infant gut microbiota in children developing allergy up to 5 years of age. Clin Exp Allergy. 2009;39(4):518-26.
9. Nermes M, Salminen S, Isolauri E. Is There a Role for Probiotics in the Prevention or Treatment of Food Allergy? Current allergy and asthma reports. 2013.
10. Johansson MA, Sjogren YM, Persson JO, Nilsson C, Sverremark-Ekstrom E. Early colonization with a group of Lactobacilli decreases the risk for allergy at five years of age despite allergic heredity. PLoS One. 2011;6(8):e23031.
11. Ozdemir O. Any benefits of probiotics in allergic disorders? Allergy Asthma Proc. 2010;31(2):103-11.
12. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet. 2001;357(9262):1076-9.
13. Elazab N, Mendy A, Gasana J, Vieira ER, Quizon A, Forno E. Probiotic Administration in Early Life, Atopy, and Asthma: A Meta-analysis of Clinical Trials. Pediatrics. 2013.
14. Campbell DE. Probiotics and primary prevention of atopic disease: Are we closer to a firm evidence base for clinical use? Clin Exp Allergy. 2013;43(9):978-80.
15. Ismail IH, Licciardi PV, Tang ML. Probiotic effects in allergic disease. Journal of paediatrics and child health. 2013;49(9):709-15.
16. West NP, Horn PL, Pyne DB, Gebski VJ, Lahtinen SJ, Fricker PA, et al. Probiotic supplementation for respiratory and gastrointestinal illness symptoms in healthy physically active individuals. Clinical nutrition (Edinburgh, Scotland). 2013.
17. Pelucchi C, Chatenoud L, Turati F, Galeone C, Moja L, Bach JF, et al. Probiotics supplementation during pregnancy or infancy for

the prevention of atopic dermatitis: a meta-analysis. Epidemiology. 2012;23(3):402-14.
18. Foolad N, Brezinski EA, Chase EP, Armstrong AW. Effect of nutrient supplementation on atopic dermatitis in children: a systematic review of probiotics, prebiotics, formula, and fatty acids. JAMA dermatology (Chicago, Ill). 2013;149(3):350-5.
19. Yang G, Liu ZQ, Yang PC. Treatment of Allergic Rhinitis with Probiotics: An Alternative Approach. North American journal of medical sciences. 2013;5(8):465-8.
20. Szajewska H. Understanding the role of probiotics and prebiotics in preventing allergic disease: evidence and methodological issues. Immunotherapy. 2013;5(8):869-78.
21. Ouwehand AC, Nermes M, Collado MC, Rautonen N, Salminen S, Isolauri E. Specific probiotics alleviate allergic rhinitis during the birch pollen season. World journal of gastroenterology: WJG. 2009;15(26):3261-8.
22. Wassenberg J, Nutten S, Audran R, Barbier N, Aubert V, Moulin J, et al. Effect of Lactobacillus paracasei ST11 on a nasal provocation test with grass pollen in allergic rhinitis. Clin Exp Allergy. 2011;41(4):565-73.
23. Koyama T, Kirjavainen PV, Fisher C, Anukam K, Summers K, Hekmat S, et al. Development and pilot evaluation of a novel probiotic mixture for the management of seasonal allergic rhinitis. Canadian journal of microbiology. 2010;56(9):730-8.
24. Costa DJ, Marteau P, Amouyal M, Poulsen LK, Hamelmann E, Cazaubiel M, et al. Efficacy and safety of the probiotic Lactobacillus paracasei LP-33 in allergic rhinitis: a double-blind, randomized, placebo-controlled trial (GA2LEN Study). European journal of clinical nutrition. 2014.
25. Moingeon P. Adjuvants for allergy vaccines. Human vaccines & immunotherapeutics. 2012;8(10):1492-8.
26. Van Overtvelt L, Moussu H, Horiot S, Samson S, Lombardi V, Mascarell L, et al. Lactic acid bacteria as adjuvants for sublingual allergy vaccines. Vaccine. 2010;28(17):2986-92.
27. Moussu H, Van Overtvelt L, Horiot S, Tourdot S, Airouche S, Zuercher A, et al. Bifidobacterium bifidum NCC 453 promotes tolerance induction in murine models of sublingual immunotherapy. Int Arch Allergy Immunol. 2012;158(1):35-42.
28. Fiocchi A, Burks W, Bahna SL, Bielory L, Boyle RJ, Cocco R, et al. Clinical Use of Probiotics in Pediatric Allergy (CUPPA): A World Allergy Organization Position Paper. The World Allergy Organization journal. 2012;5(11):148-67.

L. Giusti del Giardino[1], T. Cavallaro[1], G.P. Anzola[2], C. Lombardi[2], S. Ferrari[1]

Neuropathy in eosinophilic granulomatosis with polyangiitis: a comparison study of 24 cases with or without prior leukotriene antagonist exposure

[1]Department of Neurological, Neuropsychological, Morphological and Motor Sciences, Neuropathology Unit, University of Verona, Italy
[2]Fondazione Poliambulanza, Brescia, Italy

Summary

Eosinophilic granulomatosis with polyangiitis (EGPA), also known as Churg-Strauss syndrome (CSS), is a systemic vasculitis affecting almost exclusively patients with asthma. Neuropathy is the presenting feature in 55-75 % of cases. An increased incidence of the syndrome has been reported in asthmatics treated with leukotriene antagonists (LTAs). The causal relation is still debated. We retrospectively examined clinical, biochemical, histological features, and outcome of patients referred between 1990 and 2006 for sural nerve biopsy affected by neuropathy related to EGPA.

We identified 24 patients, 6 treated with LTA montelukast (T-group) and 18 not treated (NT-Group). All had chronic asthma; in T-group neuropathy developed from 1 to 150 days after starting montelukast. Demographic features as well as asthma duration and pre-onset treatment were remarkably similar, with the only exception of a statistically nonsignificant larger involvement of the nasal mucosa in T group. Nerve biopsy revealed in both group an axonal neuropathy. At follow-up, all within the T-group and most within the NT-group improved clinically; neurophysiological parameters remained stable, improved or worsened in the same proportion within the two groups.

Only 2 NT and no T-patient had stopped steroid treatment before the appearance of the peripheral neuropathy, making withdrawal overall unlikely as a causative factor of the onset of neuropathy. In summary, the temporal relationship between montelukast administration and the onset of neuropathy, would make the latter more likely as an "adverse drug reaction". Despite this, no significant clinical neither neurophysiological differences were noted between the two groups.

Corresponding author
Sergio Ferrari
Department of Neurological,
Neuropsychological,
Morphological and Motor Sciences
Neuropathology Unit
University of Verona
P.le L. Scuro 10, 37134 Verona, Italy
E-mail: sergio.ferrari@ospedaleuniverona.it

Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare idiopathic systemic necrotizing vasculitis described in 1951 in patients with chronic asthma who developed a pathological picture characterized by peripheral eosinophilia and granulomatous vasculitis of small and medium size vessels (1). Since the first description, this syndrome has been reported in patients without asthma, but with a much lower prevalence (2,3). The pathogenesis of EGPA remains still debated. A major role has been attributed in the past to withdraw of oral or inhaled steroids in asthmatic patients, that can unmask "forme fruste" of this syndrome following the addition of new drugs such LTI. Despite these observations, steroid withdraw cannot be considered the only triggering factor, as the syndrome has been described also in non asthmatic patients (2,3), in asthmatic patients not taking steroids (16,19), and after administration of several medications as LTAs (11,12,13,14,15,16,17,18).

According to the American College of Rheumatology criteria (4), this syndrome is diagnosed if at least 4 out of the following 6 items are present: 1) asthma; 2) peripheral eosinophils more

than 10% of white blood cell count; 3) non-fixed pulmonary infiltrates; 4) polyneuropathy or mononeuropathy; 5) paranasal sinus abnormality; 6) extra vascular eosinophils on tissue biopsy. Peripheral nervous system involvement in EGPA has been reported in 55-75% of cases, according to the series reported by Hattori (5) and Sehgal (6), and is often present at the onset of disease (7). Mononeuropathy multiplex is the most frequent pattern (it is found in 71% of patients) whereas polyneuropathy is relatively rare (29% of cases) (5).

The incidence of EGPA in general population varies from 1,8 to 3,3 cases/million/year (9). In the asthmatic population not receiving LTAs is 60 cases/million asthmatic/year (8,9). Since the advent of leukotriene antagonists (LTAs) in 1998, the incidence seems to have increased (10). All LTAs have been considered as possible triggers of this syndrome (11,12, 13,14,15,16,17,18,19).

A later population based study did not confirm the increased incidence reported previously (20). So far, just small series of patients who developed neuropathy as a sign of EGPA following the treatment with LTAs have been described in detail.

The aim of this study is to compare the peripheral involvement in patients with EGPA treated with LTAs to that in patients not treated with LTAs, and to assess whether there are differences in the neurological clinical presentation, in the neuropathological picture and in neuropathy outcome.

Material and methods

We reviewed all nerve biopsies performed in the Neuropathology Unit of the Department of Neurology of the University of Verona between 1990 and 2006. Of these, we identified the cases referred with neuropathy associated with eosinophilic granulomatosis with polyangiitis. Past medical history was obtained by reviewing medical notes. The following investigations were available: 1) full blood count and erythrosedimentation rate; 2) anti-neutrophil cytoplasm antibodies with a perinuclear pattern (pANCA); 3) chest x-ray; 4) nerve conductions studies (NCS). For some patients, results of bronchoalveolar fluid analysis, skin and muscle biopsies were also available.

Nerve conduction studies were performed in all patients according to methods described by Kimura (21). Sural nerve biopsies, performed after obtaining informed consent, were divided into three portions and processed as previously described (22). In brief, one portion was fixed in 4% paraformaldehyde, embedded in paraffin, and stained with haematoxylin-eosin; the second portion was fixed in 2.5% glutaraldehyde, embedded in epoxy resin and processed for light and electron microscopy; teased fibres were also obtained; the third portion was frozen for cryosections.

The inflammatory infiltrates were characterized by immunocytochemistry with anti-CD20 (recognizing B-lymphocyte), CD45Ro (T-cell) and anti-CD68 (macrophage) antibodies (Dako, Glostrup, Denmark) (22). Abnormalities of sural nerve biopsy were obtained by two independent neuropathologists.

Clinical and neurophysiological follow-up of each patient were performed in the referring centres. Clinical outcome was assessed by the change of the MRC score from the first to the last available neurological follow-up. Neurophysiological outcome was graded as stable (no changes in the amplitudes of compound sensory [cSAP] or motor [cMAP] action potential, or in nerve conduction velocity [NCV], or changes within normal variance), improved (increase > 20% of the amplitude of cSAP or cMAP), worsened (decrease of > 20% of the amplitude of cSAP or cMAP) or evolution to a polyneuropathy.

The patients were divided into two groups (treated or "T-group" and not treated or "NT-group"), according to whether or not they had received montelukast prior to clinical onset of neuropathy. The two groups were compared in terms of demographic and clinicopathological features, response to treatment, clinical and neurophysiological outcome. T-test with correction for multiple comparisons was used to compare normally distributed variables, and Fisher's exact test was used to compare frequencies.

Results

Clinical, laboratory and neurophysiological features

The general characteristics of the patients and the principal data of past medical history are reported in **table 1** and **2**. Twenty-four patients affected with asthma were referred to our Department after the onset of neuropathy, for diagnostic nerve biopsy. None of the patients had other possible causes of neuropathy. Six out of the 24 patients received, as therapy for asthma, montelukast (10 mg once a day) 1 to 150 days before the onset of neuropathy; in none of these patients had steroids been withdrawn, whereas in the NT group this had occurred in 2/18 (12%) patients. As for steroids, all treated patients where under inhaled steroid, while in the non-treated group three (case 1-4-5) where taking oral steroids, the others inhaled steroid. Only in one patient eosinophilia > 10% was present before neuropathy onset (this patient suffered of an hypereosinophilic syndrome). pANCA were available in 21 patients: they were positive in 3/6 (50%) within the T-group and in 6/15 (40%) within the NT-group (p = 0,523). Nasal polyposis was present in 7/18 (39%) patients within the NT group and in 5/6 (83%) within the T group, but the difference failed to reach statistical significance (p = 0,077). Radiographic chest involvement was present in 4/6 (66%) patients within the T group and in 7/16 (44%) patients within the NT group, with non-statistical difference (p = 0,635). Neurological symptoms at onset and neurophysiological findings are reported in **table 3**. Peripheral nerve involvement

presented with a mononeuropathy multiplex in 6/6 (100%) patients within the T-group, and in 15/18 (83%) in the NT-group (p = 0,285); in the remaining 3 (17%) a polyneuropathy was the pattern of nerve involvement. Upper and lower limbs were affected in both groups without any difference. Exclusive upper limb involvement was only observed in 3/18 (17%) of NT patients. Nerve conduction studies performed on admission revealed an axonal involvement in all patients and no signs of demyelination. In both groups, a sensorimotor or mainly motor involvement was dominant, while a pure or mainly sensory neuropathy was present only in 1 patient within the NT-group. In MM with a mainly motor involvement, sural nerve amplitude was not always absent but reduced, often in asymmetric pattern.

Sural nerve biopsy

Pathological findings of nerve biopsy are summarized in **table 4**. Sections stained with haematoxylin and eosin, revealed signs of vasculitis (either epineurial inflammatory infiltration or vessel wall necrosis) in 5/6 T patients (83%) and in 10/17 (59%) NT patients (p = 0,135). The infiltrate contained eosinophils in 2/6 T patients (33%) and in 4 out of 18 (22%) NT. The immunocytochemical characterization of inflammatory infiltrates showed predominantly CD45Ro positive reactive T-lymphocytes. Granuloma was not documented in any of our cases; also in literature, granuloma is rarely found in peripheral nerves. Perineurial microfasciculation was not seen in our cases. The authors looked for hemosiderin deposits in serial paraffin sections stained with HE of all nerve biopsies, but failed to find it. Semithin sections stained with toluidine blue revealed a variable loss of myelinated fibres. Signs of demyelination were not observed in teased fibres analysis. Electron microscopy confirmed that also non myelinated fibres were involved. Fibre loss was uniform in all studied fascicles in the T-group and in 10/17 (59%) in the NT-group, whereas it was focal in the remaining NT patients (p = 0,135). The degree of fibre loss was severe or moderate in 13/18 (72%) of NT patients and in 3/6 (50%) of the T-group, but the difference was not statistically significant (p = 0,742).

Treatment and follow-up

All the patients received the diagnosis of EGPA following the ACR criteria (4). Steroids were the mainstay of treatment in all patients. Cyclophosphamide was added to steroids in 4 NT and in 1 T patients. Azathioprine was never used during our time of follow-up. Length of follow-up ranged from 21 months (mean) in the T-group to 25 months in the NT-group (**table 3**). Clinical and neurophysiological follow-up were both available for all T patients, whereas in the NT group, clinical follow up was available for all patients, but neurophysiological for only 9/18 (50%). The neurological picture improved in all T patients (6/6 = 100%) while among NT patients it improved in 16/18 (89%), remained stable in 1/18 (5.5%) and worsened in 1/18 (5.5%). However, three NT patients died between 6 and 24 months after the onset of neuropathy. Death was caused by adult respiratory distress syndrome in two of them and by complications following surgery of upper respiratory tract in the third patient. Blood eosinophilia returned within normal limits in 23/24 patients.

For those NT patients for whom neurophysiological follow up was available, nerve conduction studies disclosed a stable pattern of MM in 3/9 (33%), an improvement in 3/9 (33%) and a worsening in the remaining 3/9 (33%). Likewise, in the T group 3/6 (50%) patients improved, 2/6 (33%) remained stable and 1/6 (17%) worsened (table 3).

Discussion

The pathogenesis of eosinophilic granulomatosis with polyangiitis is still debated. Although a major role has been attributed to withdraw of oral and inhaled steroids (23,29), which is reported in 88% of asthmatic patients who develop EGPA, this cannot be considered the only triggering factor, as the syndrome has been described also in non-asthmatic patients (2,3), in asthmatic patients not taking steroids (16,19) and after the administration of several medications such as macrolides (24), oestrogens (25), carbamazepine (26) and, more recently, LTAs drugs (11,12,13,14,15,16,17,18,19).

LTAs interfere with the synthesis of LTA4 from arachidonic acid by blocking 5 lipoxygenase activating protein (zafirlukast) while montelukast and pranlukast are antagonists of LTC4 and LTE4 (27). These medications have been used since 1998 to treat moderate or mild persistent asthma. The advantage in their use is the steroid sparing effect, and therefore the possibility to reduce steroid related side effects. The association between administration of LTAs and peripheral nerve involvement in patients affected by asthma, leading to florid eosinophilic granulomatosis with polyangiitis, has been reported since the release of these drugs on the market. In most of the previously described cases, however, the neuropathy appeared after steroid withdrawal, raising therefore some doubts about the role of LTAs in triggering EGPA related neuropathy (25). Just recently, an analysis of the FDA Adverse Event Reporting System seem to confirm that in most confirmed cases of EGPA, LTAs treatment is a suspect medication for triggering this syndrome (30). In the present study, among 24 patients with neuropathy and definite diagnosis of EGPA, we identified 6 cases that had previously been treated with montelukast, a leukotriene antagonist. To clarify the issue of LTAs as causative agent of EGPA neuropathy, we compared clinical, neurophysiological and peripheral nerve pathological features between the two groups, that only differed for exposure to montelukast. Unlike reported in most

small series of literature, in none of our patients previously treated with montelukast, steroid therapy was stopped before the onset of EGPA-related neuropathy, making therefore steroid withdrawal very unlikely as a causative factor. Demographic features, as well as asthma duration and systemic involvement, were remarkably similar, with the only exception of a relatively (although nonsignificantly) larger involvement of the nasal mucosa and higher systemic involvement in T patients. Presence of pANCA did not differ in the two groups. Neither clinical nor neurophysiological features seem to differ between T and non-T group: the overwhelming majority of patients presented with an axonal mononeuropathy multiplex. Axonal involvement was confirmed by sural biopsy, which revealed in all cases a variable degree of fiber loss. Signs of demyelination were not observed in teased fibers analysis. Epineurial vessel wall infiltrates of inflammatory cell (including eosinophil cells) were observed in 83% of T-cases and in 59% of NT-patients. In the T group, neuropathy onset was always preceded by systemic symptoms and the pattern of peripheral nerve involvement was a mononeuropathy multiplex. Unlike previous reports on LTAs-related neuropathy, our study was able to assess the course of the disease. Compared with non-treated patients, T-group showed a trend for a better outcome (but non-significant), as the clinical evolution was clinically more favorable in the latter (100% vs. 89% improvement) in parallel with the improvement of neurophysiologic parameters, which occurred in 50% of T but in only 33% of NT-patients despite similar treatment.

In summary, the analysis of our series suggests the following conclusions: 1) neuropathy related to EGPA does not seem to be related to steroid withdraw; this is true both in patients treated with LTAs, but also in the majority of those who had never been exposed to these medications; 2) the temporal co-relation with the administration of LTAs suggests as most obvious the interpretation of an "adverse drug reaction"; 3) no major differences between the two groups with the only exception of a relatively (although non significantly) larger involvement of nasal mucosa and a higher systemic involvement in the treated patients; 4) one or more as yet undisclosed trigger factors different from LTAs exposure or steroid withdraw might underlie the appearance of EGPA.

Strengths of the study are the consecutive recruitment of the studied cohort, the rigorous clinical and histological assessment and the ability to follow up patients. Some limitations derive from the small sample size and the inclusion criteria, which may not entirely rule out a selection bias.

Figure 1 *- Pathological features in nerve biopsies.*
A-C: Asymmetric fiber loss and axonal degenerations in nerve biopsy of case 5 (NT-group) (A) and mild fiber loss in case 19 (T-group) (C). (semithin sections, Toluidine blue).
B: Eosinophils infiltration in the wall of an epineurial vessel in case 6 (NT-group) (paraffin section, HE stain).
D: Perivascular inflammatory cell infiltration and necrosis of vesel wall in case 19 (paraffin section, HE stain)

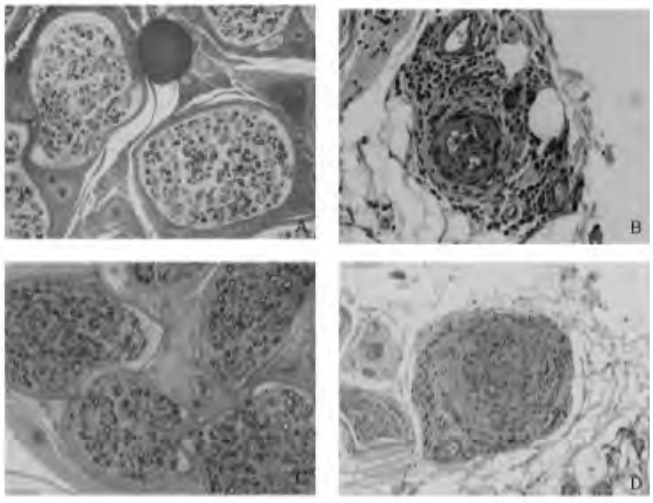


Table 1 *- General features of the patients studied.*

		ARA diagnostic criteria for CSS												
Case	**Age (years)/ sex**	**Asthma duration (years)**	**Blood eosinophils (%) at onset of symptoms**	**Paranasal sinus involvement**	**Chest involvement**	**PNS involvement**	**Tissue eosinophils**	**Other organs involvement**	**Other tissues biopsies performed & results**	**pANCA**	**Treatment for asthma before neuropathy onset**	**Steroid withdraw**	**Latency between NP onset & treatment with montelukast (days)**
1	48/F	7	53	-	-	MM	-	CNS hypereosin sy	-	na	CS βago	-	-
2	69/ M°†	<0,2	27	-	-	MM	-	muscle	-	+	CS	-	-
3	61/M	11	22	+	-	MM	-	muscle	-	na	CS βago	-	-
4	64/M†	4	6	+	+	MM	-	myelodisplastic sy	-	na	CS	-	-
5	52/M	16	20	+	na	MM	+	skin joints	-	-	CS	-	-
6	66/M	0,5	15	-	na	MM	-	aortic aneurism	-	-	βago	-	-
7	76/M	0,5	20	-	+	MM	-	skin	skin: N	-	none	-	-
8	60/F	10	50	+	+	MM	-	mucosal	skin: N	-	CS	-	-
9	46/F†	34	40	-	+	MM	+	-	-	+	CS	-	-
10	56/F	17	52	-	+	MM	+	muscle	BAL&BS: +	-	CS βago	-	-
11	62/F	4	23	-	+	PN	+	-	-	+	CS βago	-	-
12	31/F	0,6	27	-	-	PN	-	skin muscle	skin: N muscle:+	-	CS βago	-	-
13	55/F	1	65	+	-	MM	+	bowel renal	nasal polipus	+	CS βago	+	-
14	29/F	3	42	-	+	MM	+	skin	skin: + BAL&BS:+	+	CS βago	-	-
15	67/F	11	54	+	-	MM	+	-	BS:+	+	CS βago	-	-
16	69/M	6	63	+	-	MM	-	-	-	-	CS βago	+	-
17	62/F	19	33	-	-	MM	-	pericardium	-	-	CS βago	-	-
18	72/F	19	36	-	-	PN	-	-	-	-	CS	-	-
19	66/F	6	35	-	+	MM	+	-	BS:+	-	CS + M	-	2
20	49/F	7	35	+	-	MM	+	small joints	-	-	CS + M	-	48
21	46/M	6,5	28,9	+	+	MM	-	-	-	+	CS + M	-	1-3
22	59/F	19	59	+	+	MM	-	-	BAL:+	+	CS + M	-	1
23	51/M	1,5	33	+	+	MM	+	-	BS:+	+	CS + M	-	1
24	56/M	10	58	+	-	MM	-	-	skin:-	-	βago + M	-	150

F = female; M = male; PN = polyneuropathy; † = dead; MM = mononeuropathy multiplex; + = present; - = absent; na = not available; N = normal; hypereosin sy = hypereosinophilic syndrome; CNS = central nervous system; BS = bronchoscopy; BAL = bronchoalveolar lavage; + = abnormal; CS = corticosteroids; βago = βagonist; M = montelukast; ANCA = antinuclear cytoplasmic antibodies; sy = syndrome.

Table 2 *- Statistic analysis of demographic, clinical and laboratory features.*

Variable	Non Treated group	Treated group	P value
Number of patients	18	6	
Age (years)	58 ± 13 (mean +/-SD)	54.5 ± 7 (mean +/-SD)	0.533
Sex (M/F)/%	39/61	50/50	0.665
Asthma duration (years)	9.14 ± 9 (mean +/- SD)	8.33 ± 6 (mean +/-SD)	0.805
Blood eosinophil count (%) at onset of PNS involvement	36 ± 17	41 ± 13	0.488
Nasal polyposis	7/18 (39%)	5/6 (83%)	0.077
Chest involvement	4/6 (66%)	7/16 (44%)	0.635
Tissue eosinophilia	7/18 (39%)	3/6 (50%)	0.665
pANCA	6/15 (40%)	3/6 (50%)	0.523
Mean clinical follow-up (month)	25 (mean)	21 (mean)	0.628

Table 3 *- Summary of symptoms at onset, electrophysiological findings and outcome at follow up.*

Case	General symptoms before onset of neuropathy	Neurological symptoms at onset of neuropathy and limb affected	Neurophysiological findings at onset of symptoms	Clinical outcome or cause of death (follow up in months)	Neurophysiological follow-up
1	none	motor LL	MM sm LL axonal	+ sm (6)	=
2	arthromyalgia dyspnoea rhinorrea	sm UL	MM m>s UL>LL axonal	ARDS (6)	PNsm,axonal
3	arthromyalgia fever	sm ULLL	MM m>s ULLL Axonal	+ s>m (36)	-
4	fever	sm ULLL	MM sm ULLL axonal	ARDS (2)	Na
5	none	sm LL	MM m>s LL axonal	+ sm (36)	Na
6	none	sm LL, symmetric	MM m>s LL axonal	+ sm (24)	Na
7	acute dyspnoea livedo reticularis	motor LL	MM m>s U&L axonal	+ sm (36)	+

Case	General symptoms before onset of neuropathy	Neurological symptoms at onset of neuropathy and limb affected	Neurophysiological findings at onset of symptoms	Clinical outcome or cause of death (follow up in months)	Neurophysiological follow-up
8	none	s LL	MM sm ULLL axonal	+ (3)	Na
9	arthromyalgia	s LL	MM s>m LL axonal	+ sm (21)	=
10	arthromyalgia	sm LL	MM sm LL>UL axonal	- (2)	PNsm, axonal
11	fever	sm LL, symmetric	PN sm LL axonal	+ (90)	+
12	livedo, abdominal pain myalgia	s LL, symmetric	PN s, LL axonal	Na	Na
13	arthromyalgia	sm LL	MM m>s LL axonal	+ sm (60)	Na
14	fever, weight loss cramps	sm LL	MM sm LL axonal	+ (34)	+
15	fever	sm UL	MM m>s ULLL axonal	+ sm (59)	Na
16	none	s>m UL	MM sm ULLL axonal	+ sm (2)	=
17	none	sm ULLL	MM sm UULL axonal	+ sm (3)	Na
18	arthromyalgia	s ULLL	PN sm LL>UL axonal	=sm (3)	Na
19	fever, dyspnoea	sm LL	MN sm LL>UL axonal	+ sm (36)	+
20	weight loss, fever arthralgia	sm LL	MM m>s LL axonal	+s>m (25)	-s; +m
21	skin lesions	sm ULLL	MM sm ULLL axonal	+ sm (21)	=
22	headache	sm ULLL	MM s>m UL>LL axonal	+ sm (8) coma for SAH	+
23	none	s LL	MM s>m LL axonal	+sm	+
24	fever cough	sm ULLL	MM m>s UL>LL Axonal	+ m (15)	=

LL = lower limbs; UL = upper limbs; ULLL = four limbs; PN = polyneuropathy; MM = moneuropathy multiplex; s = sensory; m = motor; na = not available; ARDS = acute respiratory dystress syndrome; + = improvement; - = worsening; = = unchanged; SAH = subarachnoid haemorrhage

Table 4 *- Findings of nerve biopsy.*

Case	Degree of myelinated fibre loss	Pattern of fibre loss	Axonal degeneration	Epineurial infiltrates	Eosinophils infiltrate	Necrotizing vasculitis
1	severe	uniform	++ / -	-	-	-
2	mild	uniform	+++ / -	+	-	-
3	severe	uniform	+++ / -	-	-	-
4	very mild	focal +	++ / -	-	-	-
5	moderate	uniform	+++ / -	++	+	+
6	mild	uniform	+ / +	-	-	-
7	moderate	focal ++	+ / +	+	-	-
8	mild	uniform	++ / -	+	-	-
9	severe	focal +	++ / +	+++	+++	+
10	severe	uniform	+++ / -	-	-	-
11	severe	uniform	+++ / -	+++	-	+
12	mild	uniform	+	-	-	-
13	severe	uniform	no fibres	++	+	+
14	severe	uniform	+++ / -	+	-	-
15	mild	focal +	+ / +	+	+	+
16	severe	focal +	+++ / +	-	-	-
17	moderate	focal ++	++ / +	-	-	-
18	moderate/severe	focal +	++ / -	+	-	-
19	mild	uniform	+ / +	++	+	+
20	mild	uniform	++ / -	++	-	-
21	severe	uniform	+++ / +	+++	+	-
22	moderate	uniform	+++/-	+	-	-
23	mild	uniform	uniform	+	-	-
24	severe	uniform	+++/-	++	-	-

+/- = very mild; + = mild; ++ = moderate; +++ = severe; - = absent

References

1. Churg J, Strauss L. Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. Am J Pathol. 1951;27:277-301.
2. Chen KR, Ohata Y, Sakurai M, Nakayama H. Churg-Strauss syndrome: report of a case without pre-existing asthma. J Dermatol. 1992;19(1):40-4.
3. Chen KR, Ohata Y, Sakurai M, Nakayama H. Churg-Strauss syndrome. Non asthmatic case of Churg-Strauss syndrome with rapidly progressive glomerulonephritis. Intern Med. 1998;37(6):561-3.
4. Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP, Calabrese LH, Edworthy SM, Fauci AS, Leavitt RY et al. The American College of Rheumatology 1990 criteria for classification of Churg-Strauss syndrome (allergic granulomatosis and angitis). Arthritis Rheum. 1990;33(8):1094-100.
5. Hattori N, Ichimura M, Nagamatsu M, Li M, Yamamoto K, Kumazawa K, Mitsuma T, Sobue G. Clinicopathological features of Churg-Strauss syndrome-associated neuropathy. Brain. 1999;122(Pt 3):427-39.
6. Sehgal M, Swanson JW, DeRemee RA, Colby TV. Neurologic manifestations of Churg Strauss syndrome. Mayo Clin Proc. 1995;70(4):337-41.
7. Wolf J, Bergner R, Mutallib S, Buggle F, Grau A.J. Neurologic complications of Churg-Strauss syndrome-a prospective monocentric study. Eur J Neurol. 2010;17(4):582-8.
8. Martin RM, Wilton LV, Mann RD. Prevalence of Chug-Strauss syndrome, vasculitis, eosinophilia and associated conditions: retrospective analysis of 58 prescription-event monitoring cohort studies. Pharmacoepidemiol Drug Saf. 1999;8(3):179-89.
9. Watts RA, Lane SE, Bentham G, Scott DG. Epidemiology of systemic vasculitis: a ten-year study in the United Kingdom. Arthritis Rheum. 2000;43(2):414-9.
10. Hauser T, Mahr A, Metzler C, Coste J, Sommerstein R, Gross WL, Guillevin L, Hellmich B. The leukotriene receptor antagonist montelukast and the risk of Churg- Strauss syndrome: a case-crossover study. Thorax. 2008;63:677-82.
11. Cheng H, Leff JA, Amin R, Gertz BJ, De Smet M, Noonan N, Rogers JD, Malbecq W, Meisner D, Somers G. Pharmacokinetics, bioavailability and safety of montelukast sodium (MK-0476) in healthy males and females. Pharm Res. 1996;13(3):445-8.
12. DuMouchel W, Smith ET, Beasley R, Nelson H, Yang X, Fram D, Almenoff JS. Association of asthma therapy and Churg-Strauss syndrome: an analysis of postmarketing surveillance data. Clin Ther. 2004;26(7):1092-104.
13. Katz RS, Papernik M. Zafirlukast and Churg- Strauss syndrome. JAMA. 1998;279(24):1949; author reply 1950.
14. Wechsler ME, Garpestad E, Flier SR, Kocker O, Weiland DA, Polito AJ, Klinek MM et al. Pulmonary infiltrates, eosinophilia and cardiomiopathy following corticosteroid withdrawal in patients with asthma receiving zafirlukast. JAMA. 1998;279(6):455-7.
15. Wechsler ME, Finn D, Gunawardena E, Westlake R, Barker A, Haranath SP, Pauwels RA, Kips JC, Drazen JM. Churg-Strauss syndrome in patients receiving montelukast as treatment for asthma. Chest. 2000 Mar;117(3):708-13.
16. Green RL, Vayonis AG. Churg-Strauss syndrome after zafirlukast in two patients not receiving systemic steroid treatment. Lancet. 1999;353(9154):725-6.
17. Tuggey JM, Hosker HS, DaCosta P. Churg-Strauss syndrome associated with montelukast therapy. Thorax. 2000;55(9):805-6.
18. Kinoshita M, Shiraishi T, Koga T, Ayabe M, Rikimaru T, Oizumi K. Churg-Strauss syndrome after corticosteroid withdrawal in an asthmatic patient treated with pranlukast. J Allergy Clin Immunol. 1999;103(3Pt1):534-5.
19. Mateo ML, Cortés CM, Berisa F. Churg-Strauss syndrome associated with administration of montelukast in an asthmatic patient without underlying maintenance corticosteroid treatment. Arch Broncopneumol. 2002;38(1):56.
20. Harrold LR, Andrade SE, Go AS, Buist AS, Eisner M, Vollmer WM, Chan KA, Frazier EA, Weller PF, Weschler ME, Yood RA, Davis KJ, Plat R. Incidence of Churg-Strauss syndrome in asthma drug users: a population-based perspective. J Rheumatol. 2005;32(6):1076-80.
21. Kimura J. Principle of nerve conduction studies. In Kimura J Electrodiagnosis in disease of nerve and muscle: principles and practice. 2nd ed. Philadelphia: FA. Davis;1989a:78-02.
22. Monaco S, Lucci B, Laperchia N, Tezzon F, Currò-Dossi B, Nardelli E et al. Polyneuropathy in hypereosinophilic syndrome. Neurology. 1988;38(3):494-6.
23. Weller PF, Plant M, Taggart V, Trontell A. Workshop summary report: the relationship of asthma therapy and Churg-Strauss syndrome. NIH Workshop summary report. J Allergy Clin Immunol. 2001;108(2):175-83.
24. Roux M, Fabre M. Macrolide-induced Churg-Strauss syndrome. Eur J Intern Med. 2004;15(7):474.
25. Somogyi A, Müzes G, Molnár J, Tulassay Z. Drug-related Churg-Strauss syndrome? Adverse Drug React Toxicol Rev. 1998 Jun-Sep;17(2-3):63-74.
26. Imai H, Nakamoto Y, Hirokawa M, Akihama T, Miura AB. Carbamazepine-induced granulomatous necrotizing angiitis with acute renal failure. Nephron. 1989;51(3):405-8
27. Drazen JM, Israel E, O'Byrne PM. Treatment of asthma with drugs modyfing leukotriene pathway. NEJM. 1999;340(3):197-206.
28. Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science. 1983;220(4597):568-75.
29. Wechsler ME, Pauwels R, Drazen JM. Leukotriene modifiers and Churg-Strauss syndrome: adverse effect or response to corticosteroid withdrawal? Drug Saf. 1999;21(4):241-51.

M. Sánchez-Borges[1,2], F. Caballero-Fonseca[2], A. Capriles-Hulett [2]

Subtypes of chronic Urticaria in patients attending allergy clinics in Venezuela

[1]Allergy and Clinical Immunology Department, Clínica El Avila, Caracas, Venezuela.
[2]Allergy and Clinical Immunology Department, Centro Médico-Docente La Trinidad, Caracas, Venezuela

Key words
Angioedema; antihistamines; chronic urticaria; urticaria

Corresponding author
Mario Sánchez-Borges
Clínica El Avila, 6a transversal Urb. Altamira, piso 8, consultorio 803, Caracas 1060, Venezuela
Phone/FAX: +58 212 261 52 84
E-mail: sanchezbmario@gmail.com

Summary
Chronic urticaria (CU) is one of the most puzzling clinical entities confronted by the medical profession. It is a common motive for consultation, and in a sizable proportion of patients no identifiable cause is evident. Since there are relatively few publications regarding CU in developing countries, we performed a prospective 3-year study on the demographic and clinical features of patients with CU.
Four hundred and twenty-three subjects were studied, 52 children and 371 adults, 295 females (69.7%), with a mean age of 38.4 ± 17.8 years. More often, wheals and angioedema (AE) were present on the head, upper and lower limbs and the trunk. AE was present in 162 patients (38.4%). The most frequent subtypes were chronic spontaneous urticaria, aspirin-exacerbated cutaneous disease, dermographic urticaria, and combinations of various subtypes.
A better understanding of the characteristics of patients suffering CU is helpful for clinicians dealing with this ailment, and provides guidance for new investigations on its pathogenesis, which will hopefully result in a better management of this vexing condition.

Introduction

Chronic urticaria (CU) constitutes one of the most challenging medical conditions in allergology. It is a common cause for consultation, with lifetime prevalence rates in the general population of up to 1.8% (1). The disease compromises patient's quality of life (2,3), decreases productivity and demands substantial resources from health services (4), and in many cases does not respond to the best available treatment.

The management of patients suffering CU may be difficult due to present limitations to fully understand its pathogenesis (5), and in consequence to obtain an etiologic diagnosis, since in a large number of cases no detectable external or internal factors are the cause of the wheals, and urticaria is labelled as spontaneous.

CU affects patients from all age groups, and it may involve any areas of the skin as well as mucosal tissues in the form of angioedema (AE) of the upper respiratory and gastrointestinal tracts. When an etiologic agent is present, it is generally related to physical agents (pressure, cold, heat, sunlight, vibration), drugs, foods, contactant allergens, insect stings, or emotional stress. Among internal factors, chronic infections (for example, *Helicobacter pylori*) (6) and autoimmunity (7) are mentioned.

There are few studies in the literature on the demographic and clinical features of patients with CU, and the prevalence of CU subtypes is largely unknown, especially in developing countries. In this investigation we aimed to study prospectively patients with CU/AE observed in outpatient allergy clinics from Caracas, Venezuela, during a three-year period of observation. We regard these observations as very useful for clinicians taking care of patients suffering this common disease around the world, and more especially in developing areas of the world.

Materials and methods

This investigation is a prospective study, which included all new patients with CU of any age or sex, attending two allergy outpatient clinics in Caracas, Venezuela, between January 1st, 2010, and December 31st, 2012. Written informed consent was obtained from patients for inclusion into the study, and the protocol was approved by the Institutional Review Board from Clínica El Avila.

Data on age, gender, duration of symptoms, body distribution of wheals, triggering factors, previous and concomitant medical history, were obtained by direct questioning and physical examination done by an allergist. The following laboratory investigations and immediate-type skin prick tests with inhalant and food allergens were done in selected patients according to the information derived from the medical history and patient's examination: complete blood cell and differential leukocyte count, thyroid function tests, antinuclear and anti-thyroid antibodies, erythrosedimentation rate, C-reactive protein, serology for *H. pylori*, *Mycoplasma pneumoniae* and syphilis, total serum IgE, blood chemistry, urine and stool analysis.

CU was defined according to current International Guidelines as the appearance of wheals and/or AE lasting longer than 6 weeks (8). Subtypes of CU included spontaneous, physical, cholinergic, aquagenic, and contact urticaria. A subset of patients with chronic spontaneous urticaria (CSU) who experienced disease exacerbations when exposed to aspirin or nonsteroidal anti-inflammatory drugs that inhibit COX-1 isoenzyme of arachidonic acid metabolism (NSAIDs), were designated as having aspirin-exacerbated cutaneous disease (AECD) (9,10). Autoreactivity was confirmed in patients who showed a positive autologous serum skin test (11). Some patients exhibited combinations of various urticarial subtypes.

The treatment of CU was done following recommendations from EAACI/GA(2)LEN/EDF/WAO guidelines (12), which include second generation, nonsedating, anti-H1 antihistamines, with the addition of short courses of systemic corticosteroids for exacerbations, and leukotriene-receptor antagonists, immunosuppressants, or omalizumab in patients not responding to antihistamines at conventional or higher doses.

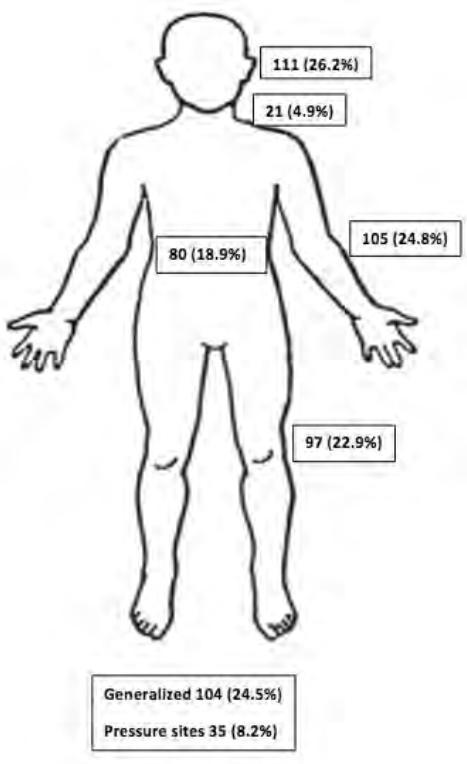
Results

During the lapse 2010-2012, 423 new patients with CU were studied. Fifty-two were children and adolescents (2 to 18 years) (12.2%) and 371 were adults (> 18 years) (87.7%), 295 females (69.7%) and 128 males (30.2%). Mean age was 38.4 ± 17.8 years (range 2-85 years) and mean disease duration was 28.2 ± 63.3 months (range 2-540 months).

Previous and concomitant medical history is shown in **table 1**, where it can be observed that most common associated diseases were allergic rhinitis, asthma, hypertension, thyroid diseases, chronic rhinosinusitis, and nephrolithiasis, while the frequency of other conditions was not increased.

Body distribution of the wheals and AE is presented in **figure 1**. Most common sites were the head (including facial AE), upper and lower limbs, and the trunk. Generalized urticaria was present in 24.5% of patients, and wheals on sites of pressure on the skin in 8.2%. AE was present in 162 patients (38.4%), with 61.4% showing exclusively urticaria, 26.0% urticaria and AE, and 12.4% AE alone.

***Figure 1** - Distribution of wheals and angioedema in 423 patients with chronic urticaria.*



The following precipitating factors were suspected from patient questioning: drugs (7.3%), foods (1.1%), and physical agents (12.9%). Less frequent inducers were emotional stress, insect stings, exercise, pets, house dust, and contact allergens (**table 2**). No specific triggers were identified by 318 patients (75.2%).

Table 3 presents the results of laboratory investigations done in 166 patients. Abnormal results were obtained in 80 (48.1%), and normal tests in 86 (51.8%). Most frequent abnormalities were blood eosinophilia, increased total serum IgE, and anti-thyroid autoantibodies. The autologous serum skin test

Table 1 *- Previous and concomitant diseases in 423 patients with chronic urticaria.*

Disease	n (%)	Disease	n (%)	Disease	n (%)
Allergic rhinitis	86 (20.3)	Diabetes mellitus	3 (0.7)	Hyperuricemia	1 (0.2)
Asthma	51 (12.0)	Fixed drug eruption	2 (0.4)	Hodgkin's disease	1 (0.2)
Thyroid disease	33 (7.8)	Atopic dermatitis	2 (0.4)	Non-Hodgkin's lymphoma	1 (0.2)
Hypertension	26 (6.1)	Chronic renal failure	2 (0.4)	Erythema nodosum	1 (0.2)
Chronic rhinosinusitis	17 (4.0)	Brain dysrhythmia	2 (0.4)	Chronic cystitis	1 (0.2)
Nephrolithiasis	11 (2.6)	Celiac disease	2 (0.4)	Psoriasis	1 (0.2)
Vitiligo	5 (1.1)	Systemic lupus erythematosus	2 (0.4)	Gilbert's disease	1 (0.2)
Migraine	4 (0.9)	Autoimmune hemolytic anemia	1 (0.2)	Hyperlipemia	1 (0.2)
Gastritis	3 (0.7)	Brain aneurism	1 (0.2)	-	-
Hyperinsulinism	3 (0.7)	Berger's disease	1 (0.2)	-	-

Table 2 *- Agents inducing wheals in 423 patients with chronic urticaria.*

Drugs (n = 31, 7.3%)		Miscellaneous (n = 69, 16.3%)		Foods (n = 5, 1.1%)	
NSAIDs	18 (4.2)	Pressure	45 (10.6)	Shellfish	3 (0.7)
ACE inhibitors	5 (1.1)	Sunlight	7 (1.6)	Milk	1 (0.2)
Radiocontrast media	2 (0.4)	Emotional stress	6 (1.4)	Fish	1 (0.2)
Oral contraceptives	1 (0.2)	Cold	3 (0.7)	-	-
Glyburide/ metformin	1 (0.2)	Exercise	2 (0.4)	-	-
Losartan	1 (0.2)	Insects	2 (0.4)	-	-
Penicillin	1 (0.2)	Cat	1 (0.2)	-	-
Lorazepam	1 (0.2)	Dog	1 (0.2)	-	-
Oxcarbazepam	1 (0.2)	House dust	1 (0.2)	-	-
-	-	Contact with cosmetic	1 (0.2)	-	-

(ASST) was done in 12 patients and all of them showed positive responses, whereas ASST was negative in 10 control individuals who did not have urticaria.

Immediate-type skin prick tests with allergens were done in 256 patients (60.5%), with positive results to at least one allergen in 157 (61.3%), and negative tests in 99 (38.6%). Cutaneous tests with *Dermatophagoides pteronyssinus* were positive in 125 patients (48.8%), *Blomia tropicalis* in 121 (47.2%), *Dermatophagoides farinae* in 54 (21.0%), American cockroach in 42 (16.4%), dog in 40 (15.6%), cat in 33 (12.8%), and moulds in 18 (7.0%). Skin tests with food extracts were positive in 36 patients (14.0%), including shellfish in 14 (5.4%), and mixed fish in 11 (4.2%) (data not shown).

The subtypes of CU in the studied population are depicted in **table 4**. Chronic spontaneous urticaria (CSU) was present in 294 patients (69.5%), followed by dermographic urticaria (14.4%). U/AE induced by drugs (excluding NSAIDs) was present in 3 patients (0.7%). In patients with CSU, 49 (16.6%) had disease exacerbations after taking NSAIDs (AECD), 12 had CAIU, and 24 (5.6%) showed combinations of various subtypes of urticaria.

Table 3 *- Abnormal laboratory results in 166 patients with chronic urticaria.*

Test	n (%)	Test	n (%)	Test	n (%)
Blood eosinophilia	19 (11.4)	Increased CRP	4 (2.4)	Increased alkaline phosphatase	1 (0.6)
Increased serum IgE	19 (11.4)	Positive serology for *H. pylori*	3 (1.8)	Increased T3	1 (0.6)
Anti-thyroid antibodies	14 (8.4)	Hyperuricemia	2 (1.2)	Decreased TSH	1 (0.6)
B. hominis in stools	7 (4.2)	Increased lactate dehydrogenase	1 (0.6)	Decreased T4	1 (0.6)
Increased ESR	6 (3.6)	Microscopic hematuria	1 (0.6)	Positive serology for syphilis	1 (0.6)
Antinuclear antibodies	5 (3.0)	Positive serology for *M. pneumoniae*	(0.6)	*A. lumbricoides* in stools	1 (0.6)
Increased aminotransferases	5 (3.0)	Proteinuria	1 (0.6)	*G. lamblia* in stools	1 (0.6)
Increased TSH	4 (2.4)	Bacteriuria	1 (0.6)	-	-

ESR: erythrosedimentation rate; TSH: thyro-stimulating hormone; CRP: C-reactive protein; T3: tri-iodothyronin; T4: Thyroxine.

Table 4 *- Subtypes of chronic urticaria.*

Subtype	n	%
Chronic spontaneous urticaria (CSU)[1]	294	69.5
Dermographic urticaria	61	14.4
Autoimmune urticaria (CAIU)	12	-
AE induced by ACE inhibitors	7	1.6
Papular urticaria	6	1.4
Solar urticaria	5	1.1
Mastocytosis	3	0.7
Cholinergic urticaria	2	0.4
Cold-induced urticaria	2	0.4
Delayed pressure urticaria	1	0.2
Contact urticaria	1	0.2
Losartan-induced AE	1	0.2
Oxcarbazepine-induced urticaria	1	0.2
Aquagenic urticaria	1	0.2
Candesartan-induced urticaria	1	0.2
Urticarial vasculitis	1	0.2
Combinations[2]	24	5.6

[1]Includes 49 patients with aspirin-exacerbated cutaneous disease (AECD). [2]Combinations: CSU and dermographic urticaria (18), CAIU and AECD (3), CSU and dermographic and cold-induced urticaria (1), CSU and solar urticaria (1), dermographic and solar urticaria (1).

Discussion

Since there are unmet needs to fully understand CU, especially in regard to its pathogenesis and management, this disease remains an important challenge for clinicians and particularly for allergists and dermatologists. We have performed this investigation in order to contribute to the knowledge of the clinical features of CU in patients seen in allergy clinics from a developing country.

Although CU may affect patients of any age or sex, in the present study it was observed more often in young adults, with predominance in women. The duration of the disease was highly variable, with some subjects suffering from recurrent wheals for many years (up to 45 years). In the subset of patients who were submitted to skin tests, it was observed that 61% were atopic, about 20% had a history of allergic rhinitis and 12% of asthma. Another associated disease common in these patients was autoimmune thyroid disease, as has been described by other authors (**table 1**) (13). AE, especially of the face and upper respiratory tract, was present in 38.4%, a proportion similar to the prevalence reported by other investigators (reviewed in reference (14)).

An environmental inducing agent was suspected only in 24.8% of patients, while in 75.2% there were no evidences suggesting a cause for symptom occurrence (**table 2**). In most cases laboratory investigations did not contribute to the diagnosis (**table 3**). This confirms the recommendations of the International Guidelines on indicating complementary tests only in selected patients, guided by the information derived from the medical history and physical examination (8).

A high proportion of our patients were labelled as having CSU. Within this group, AECD and combinations with physical urticarias were common. In a review article by Maurer et al 66-93% of patients with CU had CSU, 4-33% physical urticaria, 1-7% cholinergic urticaria, and the frequency of identification of the cause of CU varied between 0% and 43%, with a higher percentage in studies that included the ASST (14).

In the group of physical urticarias, dermographic urticaria was the most common, whereas drug-induced and food-induced urticarias were relatively infrequent. This observation is clinically important, since most patients and some physicians often believe that CU is due to food allergy. We did not explore the possibility of food additives as inducers of urticaria, because this task deserves additional study using a specially designed protocol (15). However, not all experts are convinced on the causal relationship between pseudoallergens and CU (16).

Although insect stings induce more often acute urticaria, especially in children, we observed 6 patients with chronic urticaria related to chronic exposure to mosquitoes. Papular urticaria (prurigo) is not generally included in the classification of urticaria, because in most developed countries from Europe and North America this condition is not commonly observed. It is, however, an important condition in developing countries from tropical and subtropical regions (17).

In conclusion, CU is one of the most difficult-to-manage illnesses in allergy and dermatology. We have observed that CU is more frequent in young adult females. Lesions are more often present on the head, upper and lower limbs and the trunk. AE is present in almost 40% of patients, and the most frequent CU subtypes are chronic spontaneous urticaria, aspirin-exacerbated cutaneous disease, dermographic urticaria, and combinations of various subtypes.

Present results will be helpful for clinicians in general and specialized practitioners, and will give new clues for additional investigations on the pathogenesis of CU, which hopefully will provide strategies for a better management of this condition.

References

1. Zuberbier T, Balke M, Worm M, Edenharter G, Maurer M. Epidemiology of urticaria: a representative cross-sectional population survey. Clin Exp Dermatol. 2010;35(8):869-73.
2. Maurer M, Ortonne JP, Zuberbier T. Chronic urticaria: a patient survey on quality-of-life, treatment usage and doctor-patient relation. Allergy. 2009;64(4):581-8.
3. Baiardini I, Braido F, Bindslev-Jensen C, Bousquet PJ, Brzoza Z, Canonica GW, Compalati E, Fiocchi A, Fokkens W, Gerth van Wijk R, Giménez-Arnau A, Godse K, Grattan C, Grob JJ, La Grutta S, Kalogeromitros D, Kocatürk E, Lombardi C, Mota-Pinto A, Ridolo E, Saini SS, Sanchez-Borges M, Senna GE, Terreehorst I, Todo Bom A, Toubi E, Bousquet J, Zuberbier T, Maurer M. Recommendations for assessing patient-reported outcomes and health-related quality of life in patients with urticaria: a GA(2) LEN taskforce position paper. Allergy. 2011;66(7):840-4.
4. Delong LK, Culler SD, Saini SS, Beck LA, Chen SC. Annual direct and indirect health care costs of chronic idiopathic urticaria: a cost analysis of 50 non-immunosuppressed patients. Arch Dermatol. 2008;144(1):35-9.
5. Sánchez-Borges M, Asero R, Ansotegui IJ, Baiardini I, Bernstein JA, Canonica GW, Gower R, Kahn DA, Kaplan AP, Katelaris C, Maurer M, Park HS, Potter P, Saini S, Tassinari P, Tedeschi A, Ye YM, Zuberbier T, and the WAO Scientific and Clinical Issues Council. WAO Position Paper. Diagnosis and Treatment of Urticaria and Angioedema: A Worlwide Perspective. WAO Journal. 2012;5(11):125-47.
6. Wedi B, Raap U, Kapp A. Chronic urticaria and infections. Curr Opin Allergy Clin Immunol. 2004;4(5):387-96.
7. Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. New Engl J Med. 1993;328(22):1599-604.
8. Zuberbier T, Asero R, Bindslev-Jensen C, Walter Canonica G, Church MK, Giménez-Arnau AM, Grattan CE, Kapp A, Maurer M, Merk HF, Rogala B, Saini S, Sánchez-Borges M, Schmid-Grendelmeier P, Schünemann H, Staubach P, Vena GA, Wedi B; Dermatology Section of the European Academy of Allergology and Clinical Immunology; Global Allergy and Asthma European Network; European Dermatology Forum; World Allergy Organiza-

tion. EAACI/GA(2)LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. Allergy. 2009;64(10):1417-26.
9. Sánchez-Borges M. NSAID Hypersensitivity (Respiratory, Cutaneous, and Generalized Anaphylactic Symptoms). Med Clin N Am. 2010;94(4):853-64.
10. Sánchez-Borges M, Caballero-Fonseca F, Capriles-Hulett A. Aspirin-exacerbated cutaneous disease. Immunol Allergy Clin North Am. 2013;33(2):251-62.
11. Konstantinou GN, Asero R, Ferrer M, Knol EF, Maurer M, Raap U, Schmid-Grendelmeier P, Skov PS, Grattan CE. EAACI task-force position paper: evidence for autoimmune urticaria and proposal for defining diagnostic criteria. Allergy. 2013;68(1):27-36.
12. Zuberbier T, Asero R, Bindslev-Jensen C, Walter Canonica G, Church MK, Giménez-Arnau AM, Grattan CE, Kapp A, Maurer M, Merk HF, Rogala B, Saini S, Sánchez-Borges M, Schmid-Grendelmeier P, Schünemann H, Staubach P, Vena GA, Wedi B; Dermatology Section of the European Academy of Allergology and Clinical Immunology; Global Allergy and Asthma European Network; European Dermatology Forum; World Allergy Organization. EAACI/GA(2)LEN/EDF/WAO guideline: management of urticaria. Allergy. 2009;64(10):1427-43.
13. Confino-Cohen R, Chodick G, Shalev V, Leshno M, Kimhi O, Goldberg A. Chronic urticaria and autoimmunity: associations found in a large population study. J Allergy Clin Immunol. 2012;129(5):1307-13.
14. Maurer M, Weller K, Bindslev-Jensen C, Giménez-Arnau A, Bousquet PJ, Bousquet J, Canonica GW, Church MK, Godse KV, Grattan CE, Greaves MW, Hide M, Kalogeromitros D, Kaplan AP, Saini SS, Zhu XJ, Zuberbier T. Unmet clinical needs in chronic spontaneous urticaria. A GA²LEN task force report. Allergy. 2011;66(3):317-30.
15. Akoglu G, Atakan N, Cakır B, Kalayci O, Hayran M. Effects of low pseudoallergen diet on urticarial activity and leukotriene levels in chronic urticaria. Arch Dermatol Res. 2012;304(4):257-62.
16. Kaplan AP. What the first 10,000 patients with chronic urticaria have taught me: a personal journey. J Allergy Clin Immunol. 2009;123(3):713-7.
17. Crisp HC, Johnson KS. Mosquito allergy. Ann Allergy Asthma Immunol. 2013;110(2):65-9.

C. Costa[1], P. Menesatti[1], M.A. Brighetti[2], A. Travaglini[2], V. Rimatori[1], A. Di Rienzo Businco[3], S. Pelosi[4], A. Bianchi[5], P.M. Matricardi[5,6], S. Tripodi[3]

Pilot study on the short-term prediction of symptoms in children with hay fever monitored with e-Health technology

[1]Consiglio per la Ricerca e la sperimentazione in Agricoltura, Unità di ricerca per l'ingegneria agraria, Rome, Italy
[2]Department of Biology, University of Rome "Tor Vergata", Rome, Italy
[3]Department of Pediatrics and Unit of Pediatric Allergology, Sandro Pertini Hospital, Rome, Italy
[4]TPS Production, Rome, Italy
[5]Department of Pediatrics, Mazzoni Hospital, Ascoli Piceno, Italy
[6]Department of Pediatric Pneumology and Immunology, Charité Medical University, Berlin, Germany

Key words

Allergic rhinitis; IgE, allergens; allergenic molecules; climate; meteorology; pollen; symptoms-and-medication score; forecasting models; time lag models; patient-specific models; Partial Least Squares Discriminant Analysis; electronic and technology

Corresponding author
Salvatore Tripodi
Department of Pediatrics and Unit of Pediatric Allergology
Sandro Pertini Hospital,
via dei Monti Tiburtini 389
00157 Rome, Italy
E-mail: salvatore.tripodi@gmail.com

Summary

Forecasting symptoms of pollen-related allergic rhinoconjunctivitis at the level of individual patients would be useful to improve disease control and plan pharmacological intervention. Information Technology nowadays facilitates a more efficient and easier monitoring of patients with chronic diseases. We aimed this study at testing the efficiency of a model to short-term forecast symptoms of pollen-AR at the "individual" patient level. We analysed the data prospectively acquired from a group of 21 Italian children affected by pollen-related allergic rhinoconjunctivitis and recorded their symptoms and medication "Average Combined Score" (ACS) on a daily basis during April-June 2010-2011 through an informatics platform (Allergymonitor™). The dataset used for prediction included 15 variables in four categories: (A) date, (B) meteo-climatic, (C) atmospheric concentration of 5 pollen taxa, and (D) intensity of the patient's IgE sensitization. A Partial Least Squares Discriminant Analysis approach was used in order to predict ACS values above a fixed threshold value (0.5). The best performing predicting model correctly classified 77.8% ± 10.3% and 75.5% ± 13.2% of the recorded days in the model and test years, respectively. In this model, 9/21 patients showed ≥ 80% correct classification of the recorded days in both years. A better performance was associated with a higher degree of patient's atopic sensitization and a time lag > 1. Symptom forecasts of seasonal allergic rhinitis are possible in highly polysensitised patients in areas with complex pollen exposure. However, only predictive models tailored to the individual patient's allergic susceptibility are accurate enough. Multicenter studies in large population samples adopting the same acquisition data system on smart phones are now needed to confirm this encouraging outcome.

Conflict of interest

Simone Pelosi is CEO of TPS Production; Salvatore Tripodi is a shareholder in TPS Production; Paolo M. Matricardi is receiving lecture fees from Allergopharma, Thermo Fisher Scientific and TPS Production. The rest of the authors declare that they have no relevant conflicts of interest.

Introduction

Millions of people worldwide, particularly children, suffer from allergic rhino-conjunctivitis (AR) induced by pollens (pollen-AR) (1,2). AR negatively affects patients' performance of daily activities, sleep patterns, cognitive function, work and school productivity and quality of life. Less than half of the pa-

tients regularly follow medical advice, drug therapies generally achieve only partial control of symptoms, and patient's adherence to therapy is often poor (3).

The symptoms of pollen-AR appear when the concentration of the offending pollen reaches a "threshold" value so that avoiding or reducing exposure to pollens would be useful. Awareness about airborne concentrations of pollens helps the patients and their doctors to plan effective prevention and treatment and to improve adherence to drug therapy (4). Consequently, forecasting symptoms of pollen-AR at individual level would also be useful to improve disease control (5). Unfortunately, threshold values vary not only among patients but also in the same patient during the pollen season. In fact, symptoms severity is dependent not only on pollen exposure, but also on patient's specific factors, such as living environment, the level of IgE antibodies, sensitization and simultaneous exposure to other allergenic sources, and the clinical reactivity of the target organ (eyes, nose, lungs) (6,7). Patients and doctors should be helped understanding how symptoms change during a pollen season; this may help identifying the individual co-factors facilitating symptomatic manifestations and, consequently, disease self-management. Until now, many scales, indexes or scores have been created to measure the severity of AR and the impact of this disease on the patient's daily life. The most frequently used are symptom scores (SS), medication scores (MS), and combined symptom-medication scores (SMS) (8,9).

Information Technology nowadays facilitates a more efficient and easier patient monitoring (7). Applications have been used to forecast symptoms at patient group (clustering based on pollen concentrations and allergic symptoms) (7) and - most importantly - individual level (10). The design and the development of patient-specific prediction models is a challenging task (7). The relationship between pollen counts and measures of disease severity can be simple and linear (11,12), but also very complex and non-linear (12,13). Then adequate mathematical tools (e.g. forecasts models) are necessary. Algorithms and complex models are being increasingly applied to predict trends of chronic diseases; they are rapidly evolving and their complexity is increasing with the number of variables taken into account (14,15,16). Thanks to this evolution, the performance of forecast models continues to improve in many research fields (14,15,16,17,18). Forecasting models of pollen allergies that incorporate information about the individual patient's susceptibility are moving their first steps with encouraging results (7) and - as for other chronic disease - there is room for improvement of their performance.

We aimed this pilot study at testing the efficiency of a model to short-term forecast symptoms of pollen-AR at the "individual" patient level. This model is based not only on meteo-climatic data and pollen concentrations, but also on individual risk-factors (hence the name), such as the patient's molecular profile and the overall intensity of IgE sensitization. Eventually, we analysed the data prospectively acquired from a group of children affected by hay fever and using on a daily basis and for two consecutive seasons an informatics platform (Allergymonitor™) to monitor allergic symptoms according to internationally established criteria.

Materials and methods

Study population and study design

The study population consisted of patients seeking care for pollen-AR at the Pediatric Allergy Outpatient Unit of the Sandro Pertini Hospital in Rome. Inclusion criteria were the following: A) a diagnosis of pollen-AR; B) IgE sensitization to one or more of the following four pollen sources: birch, grass, olive, pellitory, i.e. the most relevant ones in Rome between April and June (19); C) the intention to stay in Rome for the whole study period; D) lack of sensitization to perennial allergens such as *Dermatophagoides pteronyssinus*, cat, dog, *Alternaria alternata* or other molds. Each patient underwent skin prick tests for *Dermathophagoides pteronyssinus*, *Phleum pratense*, *Cynodon dactylon*, pellitory, mugwort, ragweed, cypress, birch, plane, *Olea europaea*, cat, and dog, with Histamine 0,1 mg/ml and glycerol solution as positive and negative control respectively (ALK-Abellò Milan, Italy), and a blood sample was drawn to test the concentration of IgE to major pollen allergenic molecules.

After parents or legal tutors gave a written informed consent, the patients were asked to record daily on a web-platform (AllergyMonitor©, TPS production, Rome, Italy) their symptoms and medication during the pollen season (from April 1st April to June 30th) both in 2010 and in 2011. Only patients recording symptoms and medications for > 20 consecutive days during the examination period were examined. No interpolation was applied to missing data, and only consecutive days were considered. This study was embedded in a larger epidemiological study on pollen allergy that was approved by the Ethic Committee of the Sandro Pertini Hospital (20).

Definitions

Pollen-AR was diagnosed in the presence of: (1) nasal and/or eye symptoms (apart from common cold) (21) for at least three weeks during one of the two last pollen seasons, and (2) positive SPT (wheal reaction > 3 mm) in accordance with clinical history and local pollination period. Pollen-AR was classified as mild or moderate/severe, as well as intermittent or persistent (ARIA classification) (22). The age at onset of pollen-AR was reported by the parents as their child's age as the first year with relevant symptoms. The duration of AR since its onset was established

as the difference in years between the child's age at recruitment and the child's age at AR onset. Asthma was classified as intermittent, mild persistent, moderate persistent or severe persistent (GINA classification) (23).

Symptom-Medication-Score

Patients were asked to record their symptoms and medication once a day (at evening) on Allergymonitor™. This tool is a user-friendly web based platform to monitor allergic rhinitis and asthma, accessed both via mobile (iOS and Android operative systems) or PC. For the purposes of the present study, the "Average Combined Score" (ACS) (9) has been automatically and daily calculated by the platform. We chose this score because it is derived by a combination of symptoms' score and drug therapy, so it is more reliable. The patients recorded their ocular, nasal, and bronchial symptoms as well as medication. The ACS index was calculated as previously reported (9) by combining the Rhinoconjunctivitis Total Symptom Score (RTSS) and the Rescue Medication Score (RMS) according to the following formula: [(RTSS / 6 + RMS) / 2]. The RTSS includes six individual symptoms: four nasal (sneezing, rhinorrhoea, itching, and congestion) and two ocular (itching and tearing). The intensity of each symptom can be expressed with a value from 0 to 3: 0 = absent (no sign/symptom evident); 1 = mild (sign/symptom clearly present, but minimal awareness; easily tolerated); 2 = moderate (definite awareness of sign/symptom that is bothersome, but tolerable); 3 = severe (sign/symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping). The daily total of six symptoms combined can reach a score between 0 and 18. The RMS adopts a scale based on the type of drug taken: 0 = no drug, 1 = antihistamines (topical and/or oral), 2 = nasal corticosteroids, 3 = oral corticosteroids. If two or more types of drugs are taken in a given day, the one with the highest score is taken into account. The resulting total score ranges from 0 to 3, but the score is a continuous one and its level of precision is normally at the level of 2 decimals and it is as sensitive as the other scores (24). A patient was considered to have symptoms in a given day if he/she had an ACS of at least 0.5 in that day. Allergymonitor© calculates the ACS, extracts data and automatically generates databases usable for outsource analyses.

Parameters and databases

The whole dataset (x-block) was composed of 15 variables, including (A) the ordinal dates of the year, (B) five meteo-climatic variables [temperature (maximum, minimum and mean; °C), wind speed (mean; Km h^{-1}) and rain rate (mm)], (C) the air concentration of the five most representative pollens of the study area [Betulaceae (*Betula* blooming period is May-June), Corylaceae (*Carpinus* and *Ostrya*, April-May), Graminaceae (April-June), Oleaceae (*Olea*, May-June) and Urticaceae (blooming throughout the year)], and (D) four scores based on the intensity of the patient's IgE sensitization, expressed as specific activity (see below) to the major allergenic protein of four pollens multiplied for the daily counts of the corresponding pollen (IgE-pc). These 15 variables were then used in the modeling approaches (see below). The 15 variables were used to compose three datasets progressively including an increasing number of variables (**table 1**): (1) the simplest or "meteo" dataset, composed only by ordinal dates and the 5 meteo-climatic variables (six parameters; DMC), (2) the intermediate or "meteo-pollen" dataset, including the meteo parameters and the concentration of the five pollens taken into account (overall 11 parameters; DMCP), (3) the global or "meteo-pollen-IgE" dataset, including also the four pollen-sensitization indexes (overall 15 variables; DMCPI).

Table 1 *- Database of increasing complexity used to predict trends of symptoms in 21 patients with hay fever.*

Variables	DMC	DMCP	DMCPI
Date (ordina[l])	X	X	X
Meteoclimatic1	X	X	X
Pollen concentrations[2]		X	X
IgE-pc[3]			X

1 including five variables: temperature (maximum, minimum and mean), wind speed (mean; Km h-1) and rain rate (mm)
2 including five variables: Betulaceae, Corylaceae, Graminaceae, Oleaceae and Urticaceae
3 including four variables: index of sensitization to Corylaceae, Graminaceae, Oleaceae and Urticaceae

Meteorological data and pollen counts

Both meteorological data and pollen counts were recorded at the meteorological and aerobiological station of the University of Rome "Tor Vergata", located at a distance of 10.5 km from the study center, with validated methodologies (25,26). The monitoring station aerobiological pertains to the Italian Network of Monitoring Aerobiology, R.I.M.A.®, coordinated by the Italian Association of Aerobiology® (AIA®). A volumetric sampler type Hirst (27) Model 2000 VPPS Lanzoni (28) has been used. The data acquisition is routinely carried out according to standard procedures (Standard UNI 11008:2004 - "Qualità dell'aria - Metodo di campionamento e conteggio dei granuli pollinici e delle spore fungine aerodisperse") and the pollen counts are reported as daily concentration and expressed in grains/m^3 air (26).

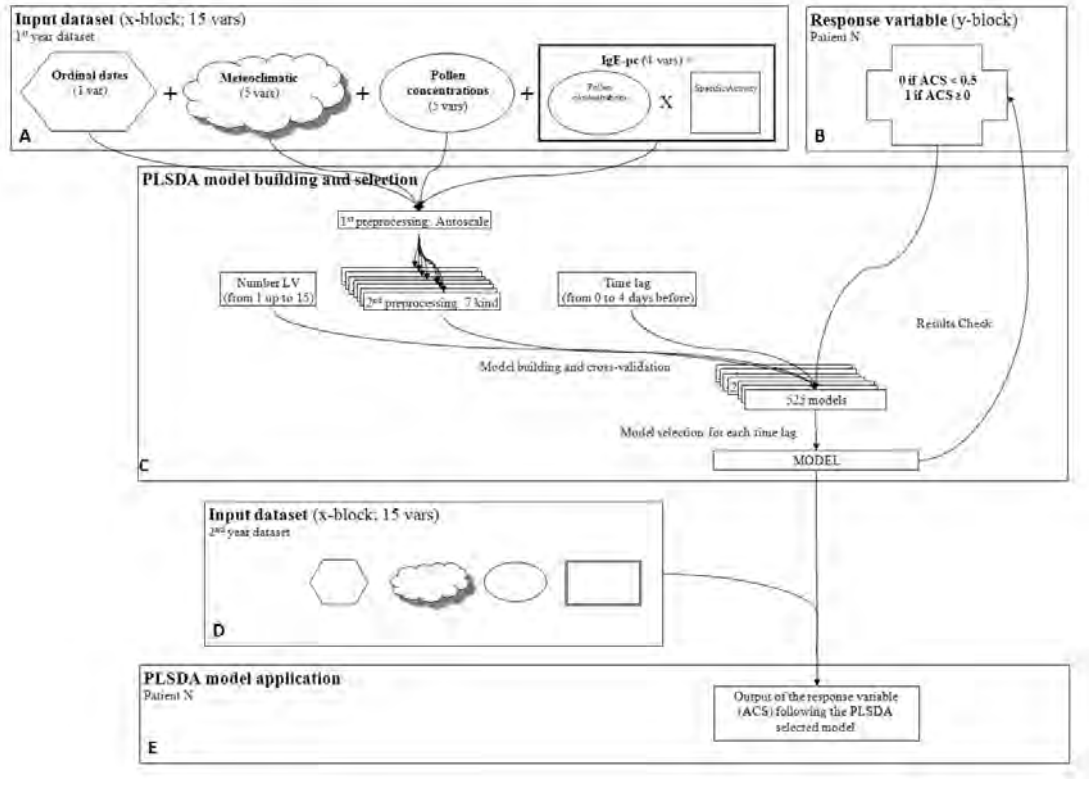
IgE assays

Total and specific IgE determination for this study have been performed as previously reported (29). IgE for allergenic molecules were tested in sera of patients showing a wheal reaction > 2 mm elicited by the corresponding allergenic source by ImmunoCAP FEIA (ThermoFisher Scientific, Uppsala, Sweden). The following major allergenic molecules were selected as previously suggested: Graminaceae (*Phleum pratense*, rPhl p1 and rPhl p5b), Oleaceae (*Olea europaea*, nOle e 1), Betulaceae (*Betula verrucosa*, rBet v1), Urticaceae (*Parietaria judaica*, rPar j2). Results were expressed in kU/L. Detection ranged from 0.35 kU/l to 100 kU/l. The IgE specific activity (SA) is the fraction (%) of patient's serum concentration of specific IgE antibodies to a given allergenic molecule within the total IgE immunoglobulins (sIgE/tIgE). For example, the SA of IgE to rPhl p1 in a patient with a level of 30 kU/l of serum IgE to rPhl p1 and a level of 360 kU/l of serum total IgE is 8,33% (30*100/360). Specific activity of IgE antibodies is a good marker for predicting the clinical response to specific allergen-specific immunotherapy (30).
An index obtained by multiplying the pollen concentration with the specific activity was also created and defined "IgE-pc" (Pollen concentration multiplied by the Specific Activity). For each patient, the value of IgE against rBet v1, rPhlp1 and rPhlp 5b, nOle e1, and rPar j2 has been multiplied by the value of the daily pollen concentration of the corresponding pollen type (Betulaceae and Corylaceae for rBet v1, Graminaceae for rPhl p1 + rPhl p5b, Olea for nOle e1, Urticaceae for rPar j2).

The multivariate time lag modeling

The proposed multivariate modeling approach predicts up to 4 days before the event the presence or the absence of symptoms (ACS > 0.5) (y-block) from an input dataset (x-block). A flowchart of the multivariate time lag modelling approach on the DMCPI dataset is summarized in **figure 1**. A Partial Least Squares Discriminant Analysis (PLSDA) approach was used in order to predict ACS values above a fixed threshold value (0.5). PLSDA consists of a classical Partial Least Squares (PLS) regression analysis where the response variable is categorical (Y-block), 0 if ACS < 0.5 (considered as absence of symptoms) or 1 if ACS ≥ 0.5 (presence of symptoms), thus expressing the class membership of the statistical units (31,32). The partitioning design consists, for each patient, in using the data from the year with the

Figure 1 *- Flowchart of the structure of the input/output dataset and the multivariate time lag modelling approach.*



larger number of records to build and cross-validate the dataset (hereafter labeled as the "model year"), and the other year as independent test (hereafter labeled as the "test year"). The prediction ability of PLSDA also depends on the number of latent vectors (LV) used in the model. The x-block was preprocessed using an autoscale algorithm (centres columns to zero mean and scales to unit variance). For the model development, a second row preprocessing step was applied. At least 7 different kind of row (second) preprocessing were applied: none, baseline (Weighted Least Squares), detrend (remove a linear trend), mean centering, msc (multiplicative scatter correction with offset), normalize (normalization of the rows) and snv (Standard Normal Deviate).
For each dataset, the best models were extracted at 5 different forecast levels (time lag) ranging from 0 to 4 days. The time lag, represents the gap between the ACS (y-block) and the x-block shifted *i* days before (16). For example at time lag = 2, using the today x-block variables the ACS relative to 2nd following day was forecasted. Therefore, for best model selection the following modeling parameters were considered: time lag (from 0 to 4), number of LVs (from 1 up to 15) and x-block second pre-processing (7). This leads to a total of 4,410 (210 for each patient) potential models for dataset DMC, 8,085 (385 for each patient) potential models for dataset DMCP and 11,025 (525 for each patient) potential models for dataset DMCPI to be elaborated. The different number of models depends on the maximum number of LV (equal to the number of the variables) that could be used. As selection rule, for each patient the 5 models (one for each time lag) with the mean higher performance value (percentage of correct classification for both model and test sets) were considered. For DMCPI model sensitivity and specificity parameters were calculated. The models were developed using a procedure written in the MATLAB 7.1 R14 environment. Difference among means has been tested using the t-test, $p < 0.05$ was considered significant. Normality has been tested with the Shapiro-Wilk test. Confidence intervals have been expressed as standard deviation (SD) or standard error (SE).

Observation period and missing values

The period used to build the model was 70.7 ± 15.8 days (range 30-90) and the period used to test the model was 44.1 ± 21.0 days (range 16-90).

Results

Characteristics of the study population

In all, 29 patients were recruited, but only 21 (72%) completed the study. Reasons for drop-out were: unplanned moving (n = 2) and too short period of registration (< 20 days of registration) in 2010 (n = 5) and in 2011 (n = 1). The characteristics of the patients completing the study are reported in **table 2**. In most of the patients, allergic rhinitis had started 3 or more years before (average disease duration 6.1 ± 0.8 years). The average age at disease onset was 4.6 ± 0.5 years. Overall, 12/21 patients had also bronchial asthma. All the patients had serum total IgE levels > 150 kU/l and specific IgE antibodies to at least one of the tested major pollen allergenic proteins (**table 2**).

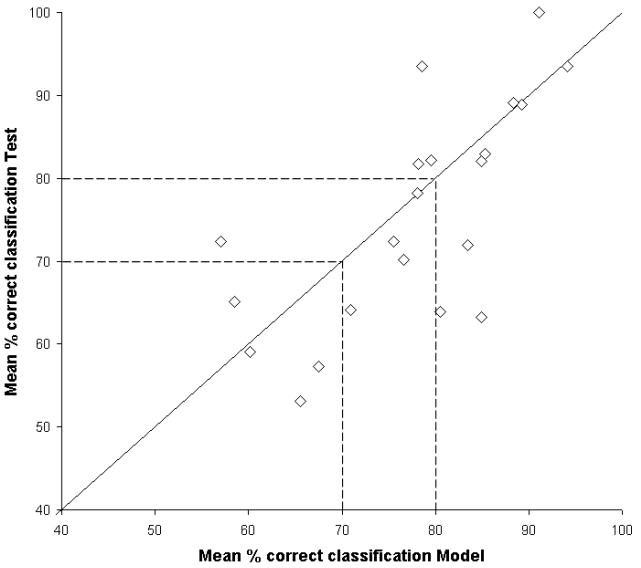
Observation period and missing values

During the year used to build the model, a higher mean number of days with ACS > 0.5 (49.6 ± 22.9 vs. 22.7 ± 21.4) (t-test; $p < 0.001$) was observed (**table 3**).

Performance of the three predictive models

The predictive performance of the models developed on the three datasets (DMC, DMCP, DMCPI) progressively improved with the dataset size (**table 4**). The best performing dataset (DMCPI) correctly classified 77.8% ± 10.3% and 75.5% ± 13.2% (p 0.21) of the recorded days in the model and test years, respectively. In this predicting approach, 9/21 patients (42.9%) showed ≥ 80% correct classification of the recorded days in both years. The **figure 2** shows the models' performance using the DMCPI dataset. On the DMCPI dataset, the mean sensitivity and specificity parameters, for both model and test years, resulted to be high (78.2 ± 13.4 and 74.9 ± 14.2 respectively).

Figure 2 *- Mean percentage of correct classification for each patient for both model and test sets using the DMCPI dataset.*



The model year performance seems to be related to that of the test year. Data are normally distributed (Shapiro-Wilk $p > 0.05$). The

Table 2 *- Characteristics of the study population°.*

	mean	SE	n	%
Age (years)	11.7	0.7		
Allergic rhinitis				
Age of onset (y)	4.6	0.5		
Duration (y)	6.1	0.8		
ARIA classification (severity)§				
mild				
intermittent (n,%)			1	4.8%
persistent (n,%)			5	23.8%
moderate/severe				
intermittent (n,%)			8	38.1%
persistent (n,%)			7	33.3%
Asthma				
Age of onset (y)	4.8	0.8		
Duration (y)	4.7	0.9		
GINA classification (severity)§*				
absent (n,%)			9	42.8%
intermittent (n,%)			10	47.6%
persistent mild (n,%)			1	4.8%
persistent moderate/severe (n,%)			1	4.8%
IgE responses*				
Total IgE (kU/l)*	499.8	89.0		
rBet v 1**	2.3	1.5		23.8%
rPhlp1**	29.8	4.6		95.2%
rPhlp5b**	34.1	20.1		76.2%
nOle e 1**	6.2	14.7		76.2%
rPar j 2**	37.5	18.6		23.8%
sum IgE-SA*	0.1	0.0		

° 21 participants, 12 (57.1%) males
§ n and % of patients with moderate/severe symptoms (criterion to classify the severity of allergic rhinitis - AR)
§* n and % of patients with persistent moderate/severe symptoms (criterion to classify the severity of allergic rhinitis - AR)
* geometric means and standard errors
** geometric means and standard errors on positive values

best performing patients tended to a higher degree of atopy, i.e. to a higher number of pollen sensitizations (figure 2,3). Similarly, poor prediction performances (< 70%) were observed among the patients with a lower degree of atopy, characterized by IgE-SA < 0.15 (data not shown). A time lag > 1 tended to be associated with better performances and no significant differences were observed between model and test year at each time lag (**table 5**). In **figure 4** the forecasting results of each single patient's PLSDA model at time lag 4 on the DMCPI dataset during the month of May of the testing year were reported. It is possible to observe the high accuracy of each patient specific model and the different patient-specific day of passing the threshold value.

Figure 3 *- Mean percentages of correct classification for each patient for both model and test sets using the DMCPI dataset. Error bars indicate SE. t-test was used to compare model and test percentages (* p < 0.05).*

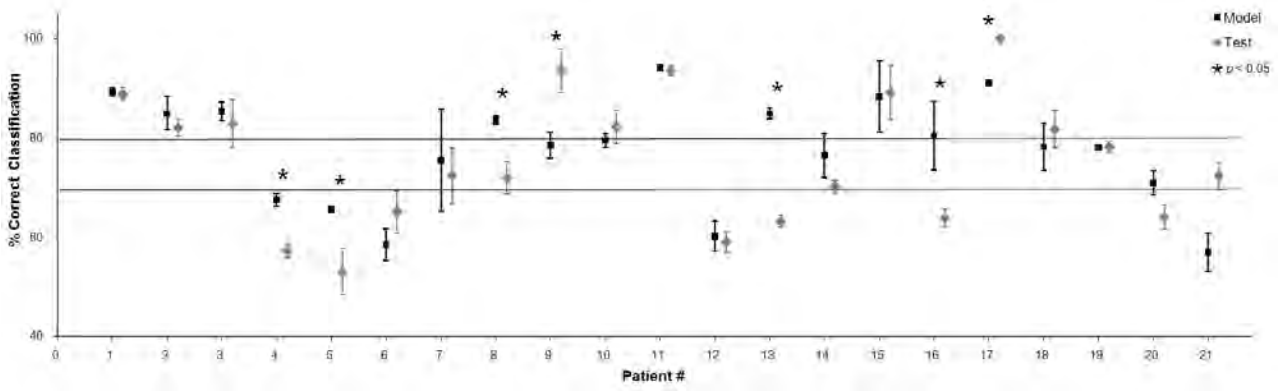


Table 3 *- Recorded data during the Model and the Test period.*

	Model		Test		p°
	mean	**SD**	**mean**	**SD**	
Consecutive recorded days	70.7	15.8	44.1	21.0	> 0.0001
Days with symptoms*	49.6	22.9	22.7	21.4	> 0.0001

* a cut-off of ACS> 0.5 has been used for positivity
° t-test comparing model and test percentages

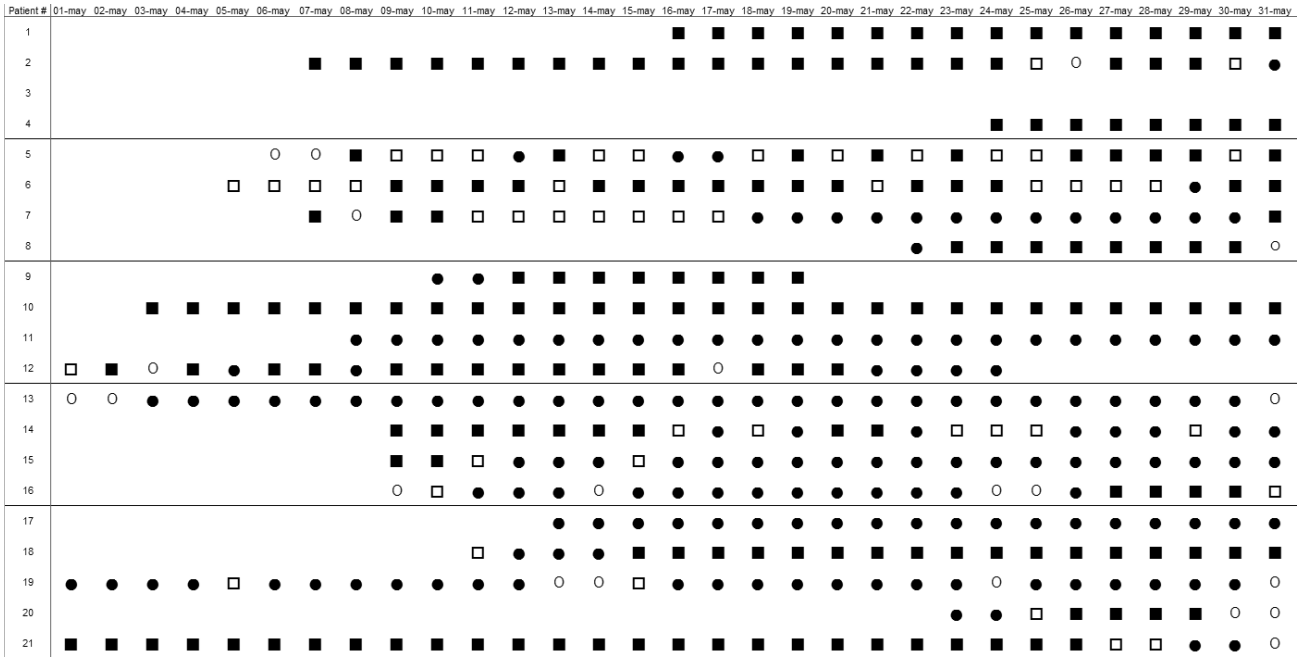
In the upper left box (A), the input datasets (x-block) composed by the 15 variables were represented by 4 different shapes identifying the 4 different groups of variables: ordinal dates (1 variable), meteoclimatic (5 variables), pollen concentrations (5 variables) and IgE-pc (4 variables). In the upper right box (B) the response variable (y-block) was represented as a cross shape. The central box (C) summarizes the PLSDA model building and selection using, for each patient, the model year dataset; the x-block was preprocessed using an autoscale algorithm, then a second row preprocessing step was applied using seven different algorithms: none, baseline (Weighted Least Squares), detrend (remove a linear trend), mean centering, msc (multiplicative scatter correction with offset), normalize (normalization of the rows) and snv (Standard Normal Deviate). For each dataset, the best models were extracted at 5 different forecast levels (time lag) ranging from 0 to 4 days and 15 Latent vectors (from 1 up to 15). This leads to a total of 4,410 (210 for each patient) potential models for dataset DMC, 8,085 (385 for each patient) potential models for

Table 4 *- Performances of the best algorithm, by database used for calculation, in 21 patients with hay fever.*

	DMC			DMCP			DMCPI		
	Model	**Test**	**p°**	**Model**	**Test**	**p°**	**Model**	**Test**	**p°**
Average performance (mean, SD)	70.1	54.8	> 0.0001	76.1	66.2	> 0.0001	77.8	75.5	0,035
	16,8	17.5		8.9	8.7		10.3	13.2	
# Patients ≥ 80% pcc* (%)	38	5		38	5		43	43	
70 ≤ # Patients < 80% pcc* (%)	5	19		38	38		33	24	
# Patients ≤ 70% pcc* (%)	57	76		24	57		24	33	

°t-test comparing model and test percentages
*pcc: percentage of correct classification

Figure 4 - *Forecasting results of the single patient's PLSDA model at time lag 4 on the DMCPI dataset during the month of May of the test year.*



Black squares = ACS ≥ 0.5, correctly classified; white squares = ACS ≥ 0.5, un-correctly classified; black circles = ACS < 0.5, correctly classified; white circles = ACS < 0.5, un-correctly classified.

dataset DMCP and 11,025 (525 for each patient; reported in the scheme) potential models for dataset DMCPI to be elaborated. The application of the selected models on the second year dataset was summarized in the box D using the different shapes used in box A. The input dataset in box D, applying the model selected in box C, extracted for each time lag, reports a provisional output of the ACS values as below or above 0.5 (box E).

Table 5 - *Influence of the time lag on the algorithm prediction performance in 21 patients with hay fever (DMCPI dataset).*

	Model pcc*		Test pcc*		p
	mean	SD	mean	SD	
0	78.3	12.9	73.1	14.7	0.23
1	75.8	12.1	72.8	13.8	0.45
2	80.4	14.6	77.5	15.1	0.53
3	78.2	79.1	79.1	13.1	0.60
4	76.9	12.3	75.3	16.3	0.72

* pcc: percentage of correct classification
° t-test

Discussion

In a prospective study of children with hay fever, we tested the symptom predictive accuracy of forecast models of increasing complexity. We found that a multivariate modeling approach can accurately predict the presence or absence of symptoms up to 4 days before the event. We also found that the models' predictive performance tended to improve when the degree of individual allergic susceptibility was also taken into account. Finally, we found that the predictive performance improves at time lag values > 1 day after exposure.

The performance obtained by our model is relatively good, and comparable to the one obtained in previous studies. The symptoms of seasonal allergic rhinitis in 102 adult Austrian patients were recently predicted with a model based on day number of the year, grass pollen counts of the previous 2 weeks, forecasted grass pollen counts, maximum and mean temperatures (7). This model had a similar performance (76%) as the one (78%) obtained in our Italian children. Similarly, de Weger et al. (33) predicted the severity of symptoms in 80 (Netherlands) adults with hay fever with a model based on three risk categories and a time-lag of 1-5 days. The prediction performance ranged between 65% and 77% (33). Interestingly, our model - although

tested in areas with higher aerobiological complexity and in highly polysensitized patients - provided similar performances as the Austrian and Netherlands models.

The reasons of such a good performance deserve further discussion. First, our model has some strength in its statistical approach, when compared to the previous ones: it was tested by comparing data acquired in different years, rather than within the same years. The use of an external control (model vs. tested year) is considered more robust (34,15,16) and can increase the reliability of the prediction. Second, our model was tailored on the patient's sensitization profile detailed at molecular level thanks to IgE testing, while other models have been considering only patient-independent environmental components. A personalized forecast of symptoms has been recently proposed (35), but it was based on the patient' threshold of sensitivity (i.e. a subjective parameter). Conversely, the evaluation of patient's susceptibility is in our model based on the objective evaluation of his/her sensitization profile, detailed at molecular level and related to the total IgE levels (30,35,36). Our model can therefore strengthen or weaken the impact of the pollen count variables on the basis of their clinical relevance in the individual patient. Third, the best performance was obtained by using a > 2 days lag time between pollen counts and patient's symptoms. This observation is in agreement with previous studies (33), the widely accepted concept of a delay between meteorological modifications and their consequences on pollination (16) and a delay or cumulative impact of pollen exposure on symptoms (13).

Our approach is a good basis for further developments. The proposed method has been engineered using a partitioning based on the 2 years of recorded data by each patient. An adaptive routine, based on the same PLSDA multivariate approach, may auto-train and update the model at each new daily record, thus adding new data to the historical ones. The possibility to have different time lag models will also allow forecasting symptoms up to one week. Patients often do not follow a daily therapy, and an early prevision of symptoms could improve the adherence. This kind of approach, when automatically integrated with pollen observatory and meteo-climatic stations, could be implemented on mobile devices to return the patient online feedback. Therefore, the allergic symptoms forecast may aid allergy sufferers to avoid exposure to atmospheric concentrations of allergenic pollen, and help them plan taking medication, always under medical supervision (37). Hay fever is not a disease severe enough to generate excessive anxiety and, moreover, is one of those diseases whose level of symptoms can be modulated by changing behavior (e.g. by improving adherence to medication or by reducing allergen exposure). We therefore believe that predicting symptoms can improve the patient's self-confidence and disease self-management.

We have to acknowledge some limitation in our study. First, that the population sample examined in this pilot study is relatively small and that our conclusions would have been more solid if based on a larger dataset in the next future. Moreover, the informatics platform used in our study runs, partially, on a normal computer, an approach that may be considered old fashioned. This platform is however now available on smart phones. Third, patients with less than 20 recording days were not included in the analysis, but these patients have usually a lower compliance and a worse symptoms control. In a future larger sized study, our prediction tool should be evaluated also for these patients. Fourth, the population setting included only children and the conclusion of this study need to be reproduced in an adult population. Similarly, the generalizability of our conclusions is geographically limited, and studies with the same approach should be done in area with different climatic and aerobiological conditions. Moreover, the ACS threshold (0.5) values, which indicates moderate symptoms, could seem of less importance for patient life, but *i.* we observed different patient-specific days of passing the threshold value (**figure 4**), indicating a model behavior tailored on each patient and *ii.* the possibility to use other ACS threshold values for more severe symptoms, when the dataset size will be increased (in this study too few patients showed severe symptoms). The proposed approach could be applied to other Medication Scores.

In conclusions, this monocentric study in a small population shows that symptom forecasts of seasonal allergic rhinitis is possible also in highly polysensitised patients in geographic areas with complex pollen exposure, provided that predicting models are made precise as possible by tailoring their algorithms to the individual patient's allergic susceptibility. Future studies will have to monitor how e-diaries and predictive algorithms can influence adherence to treatment, at the extremes and during the peak of the pollen season. Multicenter studies in large population samples adopting the same acquisition data system on smart phones are now needed to confirm and reinforce this encouraging conclusion.

References

1. Asher MI, Montefort S, Björkstén B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet. 2006;368:733-43.
2. Brozek JL, Bousquet J, Baena-Cagnani CE, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 Revision. Journal of Allergy and Clinical Immunology. 2010;126:466-76.
3. Canonica GW, Bousquet J, Mullol J, Scadding GK, Virchow JC. A survey of the burden of allergic rhinitis in Europe. Allergy. 2007;62(suppl 85):17-25.
4. Emberlin J, Mullins J, Cordon J, et al. Regional variations in grass pollen seasons in the UK, long term trends and forecast models. Clinical and Experimental Allergy. 1999;29:347-56.

5. Peden D, Reed CE. Environmental and occupational allergies. Journal of Allergy and Clinical Immunology. 2010;125:S150-60.
6. Traidl-Hoffmann C, Jakob T, Behrendt H. Determinants of allergenicity. Journal of Allergy and Clinical Immunology. 2009;123:558-66.
7. Voukantsis D, Karatzas K, Jaeger S, Berger U, Smith M. Analysis and forecasting of airborne pollen–induced symptoms with the aid of computational intelligence methods. Aerobiologia. 2013;29:175-85.
8. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy. 2008;63(Suppl 86):8-160.
9. Häfner D, Reich K, Matricardi PM, Meyer H, Kettner J, Narkus A. Prospective validation of 'Allergy-Control-SCORETM': a novel symptom–medication score for clinical trials. Allergy. 2011;66:629-36.
10. Berger U, Karatzas K, Jaeger S, Voukantsis D, Sofiev M, Brandt O, et al. Personalized pollen−related symptom−forecast information services for allergic rhinitis patients in Europe. Allergy. 2013;68:963-5.
11. D'Amato G, Cecchi L, Bonini S, et al. Allergenic pollen and pollen allergy in Europe. Allergy. 2007;62:976-90.
12. Moreno-Grau S, Elvira-Rendueles B, Moreno J, et al. Correlation between Olea europaea and Parietaria judaica pollen counts and quantification of their major allergens Ole e 1 and Par j 1-Par j 2. Annals of Allergy, Asthma & Immunology. 2006;96:858-64.
13. Feo Brito F, Mur Gimeno P, Carnes J, et al. Olea europaea pollen counts and aeroallergen levels predict clinical symptoms in patients allergic to olive pollen. Annals of Allergy, Asthma & Immunology. 2011;106:146-52.
14. Astel A. Chemometrics based on fuzzy logic principles in environmental studies. Talanta. 2007;72:1-12.
15. Menesatti P, Antonucci F, Costa C, Mandalà C, Battaglia V, La Torre A. Multivariate forecasting model to optimize management of grape downy mildew control. Vitis. 2013;52:141-8.
16. Brighetti MA, Costa C, Menesatti P, Antonucci F, Tripodi S, Travaglini A. Multivariate statistical forecasting modelling to predict Poaceae pollen critical concentrations by meteoclimatic data. Aerobiologia. 2014;30:25-33.
17. Abraham B, Ledolter J. (2009). Statistical methods for forecasting. John Wiley & Sons, 2009.
18. Doganis P, Alexandridis A, Patrinos P, Sarimveis H. Time series sales forecasting for short shelf-life food products based on artificial neural networks and evolutionary computing. Journal of Food Engineering. 2006;75:196-204.
19. Travaglini A, Mazzitelli A, Leonardi D, Grilli Caiola M. 2002. Monitoraggio di polline urbano e allergie. In: Atti dell'Accademia dei Lincei Conferenza annuale della Ricerca: Ecosistemi urbani 2002;1-40. ISBN/ISSN:0391-805X.
20. Dondi A, Tripodi S, Panetta V, et al. Pollen-induced allergic rhinitis in 1360 Italian children: comorbidities and determinants of severity. Pediatric Allergy and Immunology. 2013;24:742-51.
21. Asher MI, Keil U, Anderson HR, et al. International study of asthma and allergies in childhood (ISAAC): rationale and methods. European Respiratory Journal. 1995;8:483-91.
22. Jáuregui I, Dávila I, Sastre J, et al. Validation of ARIA (Allergic Rhinitis and its Impact on Asthma) classification in a pediatric population: the PEDRIAL study. Pediatric Allergy and Immunology. 2011;22:388-92.
23. Bousquet J, Clark T, Hurd S, et al. GINA guidelines on asthma and beyond. Allergy. 2007;62:102-12.
24. Grouin J-M, Vicaut E, Jean-Alphonse S, et al. The average Adjusted Symptom Score, a new primary efficacy end-point for specific allergen immunotherapy trials. Clinical & Experimental Allergy. 2011;41:1282-8.
25. UNI. Qualità dell'aria - Metodo di campionamento e conteggio dei granuli pollinici e delle spore fungine aerodisperse. UNI 11008, 2004.
26. Travaglini A., Albertini R., Zieger E., Brighetti M.A., Ugolotti M., Torrigiani T. (Eds.). Manuale di gestione e qualità della rete italiana di monitoraggio in Aerobiologia R.I.M.A.®. Bologna Tipografia LEGO, 2009. ISBN 978-88-900277-1-0.
27. Hirst JM. Changes in atmospheric spore content: Diurnal periodicity and the effects of weather. Transactions of the British Micological Society. 1952;36:375-93.
28. Mandrioli P. Metodica di campionamento e conteggio dei granuli pollinici e delle spore fungine aerodisperse. In Regione Emilia Romagna (Ed.) Il monitoraggio aerobiologico in Emilia Romagna. 1994;30:9-19.
29. Stringari G, Tripodi S, Caffarelli, et al. The effect of component resolved diagnosis on specific immunotherapy prescription in children. Journal of Allergy and Clinical Immunology. 2014;134:75-81
30. Di Lorenzo G, Mansueto P, Pacor ML, et al. Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy. Journal of Allergy and Clinical Immunology. 2009;123:1103-10.
31. Sjöström M, Wold S, Söderström B. PLS Discriminant Plots, Proceedings of PARC in Practice. Elsevier Science Publishers B.V. North-Holland, 1986.
32. Costa C, Antonucci F, Boglione C, Menesatti P, Vandeputte M, Chatain B. Automated sorting for size, sex and skeletal anomalies of cultured seabass using external shape analysis. Aquacultural Engineering. 2013;52:58-64.
33. de Weger LA, Beerthuizen T, Hiemstra PS, Sont JK. Development and validation of a 5-day-ahead hay fever forecast for patients with grass-pollen-induced allergic rhinitis. International Journal of Biometeorology. 2013;1-9.
34. Adams-Groom B, Emberlin J, Corden J, Millington W, Mullins J. Predicting the start of the birch pollen season at London, Derby and Cardiff, United Kingdom, using a multiple regression model, based on data from 1987 to 1997. Aerobiologia. 2002;18:117-23.
35. Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. Journal of Allergy and Clinical Immunology. 2008;122:298-304.
36. Hamilton RG, MacGlashan DWJr, Saini SS. IgE antibody-specific activity in human allergic disease. Immunologic Research. 2010;47:273-84.
37. Berger U, Karatzas K, Jaeger S, et al. Personalized pollen-related symptom-forecast information services for allergic rhinitis patients in Europe. Allergy. 2013;68:963-5.

Eur Ann Allergy Clin Immunol Vol 46, N 6, 226-228, 2014

A. Graziani[1], O. Quercia[2], F. Girelli[3], A. Martelli[1], F. Mirici Cappa[1], G.F. Stefanini[1]

Omalizumab treatment in patient with severe Asthma and Eosinophilic Granulomatosis with Polyangiitis. A case report

[1]UO Medicina Interna, Ospedale degli Infermi, Faenza, Italia
[2] Ambulatorio Alta Specializzazione in Allergologia, Ospedale degli Infermi, Faenza, Italia
[3] Servizio di Reumatologia Ospedale GB Morgagni, Forlì, Italia

Key words

Eosinophilic Granulomatosis with Polyangiitis (EGPA); Asthma; Omalizumab; eosinophilia

Corresponding author
Alessandro Graziani
Medicina Interna, Ospedale Infermi
Faenza, Italy
AUSL Ravenna
Phone: +39 054 660 12 14
E-mail: alessandro.graziani@ausl.ra.it

Summary

Eosinophilic Granulomatosis with Polyangiitis (EGPA), formerly named Churg Strauss Syndrome, is a multisystem disorder characterized by chronic rhinosinusitis, asthma, and prominent peripheral blood eosinophilia; it is classified as a vasculitis of the small and medium sized arteries, although the vasculitis is often not clinically apparent in the initial phases of the disease. We present the case of a woman with EGPA who was frequently treated with high dose steroid therapy during hospital admission for refractory asthma. After December 2008, the date when we started Omalizumab, we observed a significative reduction of circulating eosinophils and IgE serum level, and the patient was no more hospitalized for respiratory failure or asthma attacks.

Introduction

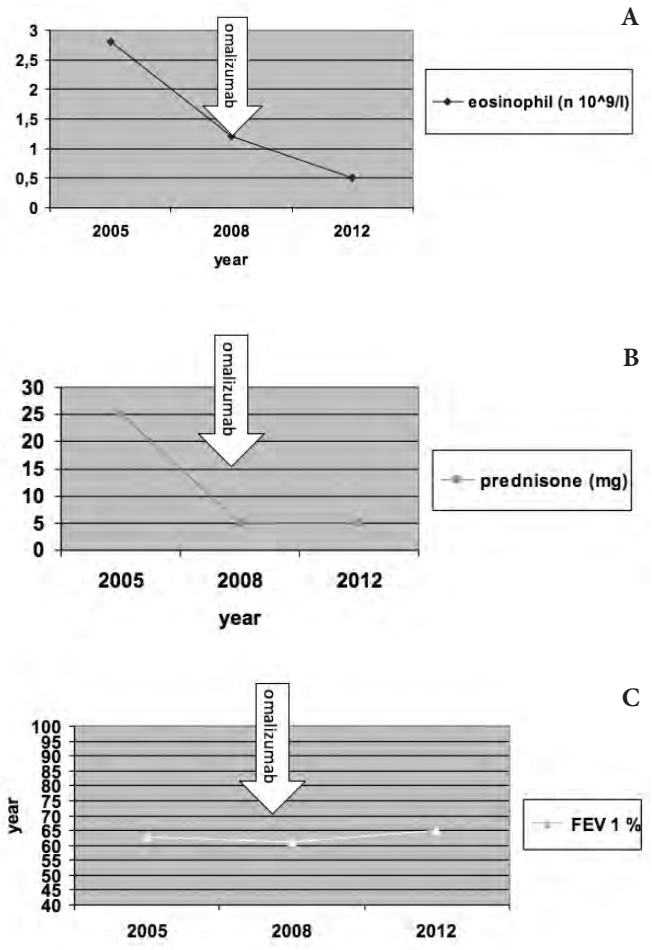
Eosinophilic Granulomatosis with Polyangiitis is a systemic disease characterized by small-medium vessels vasculitis, asthma, peripheral blood eosinophilia and constitutional symptoms with a morbidity rate of 2.4-6.8/million among the general population; necrotizing lesions of the upper airway are not frequently observed. It was first distinguished from classic Polyarteritis Nodosa in 1951 by Jacob Churg and Lotte Strauss (1,2). The disease typically affects patients between 40 and 60 years (mean 48), many of which having a prodromal stage with atopic disease in the upper respiratory tract, followed by asthma and features of cutaneous vasculitis, i.e. subcutaneous nodules, purpura, erythematous rash (3). Asthma often improves when treatment for EGPA is started (4), but some patients may persistently complain of an airflow obstruction due to uncontrolled asthma, despite the immunosuppressive treatment and the clinical remission of EGPA (5). We describe the case of a woman with a five years history of allergy to dust mites, asthma and EGPA, who was frequently treated with high dosage corticosteroids during the admissions for refractory asthma and in which the introduction of Omalizumab induced a reduction of asthma attacks, peripheral eosinophilia and IgE levels, even if corticosteroids were tapered to very low dosage.

Case report

In 2004, a 56 years old female with an history of atopic rhinitis seldom complicated by mild asthma attacks and high level of IgE specific for grass and dust mites, had a sub-arachnoid hemorrhage due to ruptured aneurysm of anterior communicating artery. A complete neurological recovery was reached after several months of rehabilitation. Five months after discharge, she was hospitalized for the first severe asthma attack. Laboratory

disclosed: erythrocyte sedimentation rate 45 mm/1°h, C-reactive protein 14.5 mg/dl (< 0,5), hemoglobin 12.0 g/dl, white blood cell count 12.7 10^9/l with 22% eosinophils (calculated eosinophils were 2.8 10^9/l), platelet count 561 10^9/l. Other routine biochemical tests, including renal and liver tests, C3 and C4 complement factors, protidogram, and Immunoglobulin G/A/M levels were normal. Blood cultures, urinalysis, serologic test for viral and bacterial infections (cytomegalovirus, influenza viruses, HCV and HBV, Mycoplasma pneumoniae and Chlamydia pneumoniae, Legionella) were negative. Among non organ-specific autoantibodies, we found high levels of ANCA-MPO with 246.4 IU/l (< 7), while cyclic citrullinated peptide antibodies, ANA test, anti-ENA, anti-Cardiolipins, LAC, and cryoglobulins were absent. Serum IgE levels were elevated: 3365 IU/ml (N < 20). Due to the presence of upper and lower paresthesias, we performed an electromyogram, which showed features of mononeuritis multiplex involving peroneal and sural nerves at the right side. Lung CT-scan showed bilateral patchy non-cavitated pulmonary infiltrates; bronchoalveolar lavage demonstrated a high number of eosinophils (30%), excluding bacterial, mycobacterial and fungal infection. An airflow limitation with improvement after administration of inhaled bronchodilator was revealed by spirometry (**figure 1**, **panel C**). A diagnosis of EGPA was made. In the light of reported family history of breast cancer, we chose parenteral prednisone (1 mg/kg/day) as an immunosuppressive treatment leading to a progressive clinical and radiological improvement. Patient was discharged with the indication to take oral prednisone, inhaled fluticasone and formoterol at daily dosages of 25 mg, 1000 mcg and 24 mcg per day respectively. In 2006 and 2007 she was admitted many times at our Internal Medicine Unit for severe recurrent asthma attacks, requiring high doses of steroid therapy (up to 1 mg/kg/day methylprednisolone). In December 2008, on the basis of some clinical evidence, treatment with Omalizumab (150 mg every two weeks) was started. A significant persistent reduction of IgE levels, p-ANCA and peripheral eosinophil levels was observed; oral and inhalatory steroid doses were progressively reduced to 5 mg/day of prednisone and 250 mcg/day of fluticasone, with no more asthma attacks. Over the next five years of therapy the patient was no longer hospitalized for asthma attacks or respiratory failure. Despite the respiratory improvement, the peripheral nerve complications and the FEV 1 value were unaffected.

Figure 1 *- Time course of eosinophil count, steroid dose and FEV1.*
Panel A, B, C: X values= years
Panel A: Y values= eosinophil count (number/l)
Panel B: Y values= prednisone doses (mg)
Panel C: Y values= FEV1 in percentage of predicted values



Discussion

The prominent clinical problem of our patient were frequent asthma attacks, with a family history of cancer that made us reluctant to prescribe immunosuppressive drugs other than steroids. The episodes required frequent hospital admissions, and consequent high IV doses of prednisone. Omalizumab is effective in allergic asthmatic patients (8) with no report of malignancy. Some trials of Omalizumab treatment of asthma in EGPA patients, not fully responders to immunosuppressive regimen, have been published with conflicting results (6,7,10,11). In particular Giavina-Bianchi (7) and Bargagli (10) described positive outcomes with this treatment, while Ruppert (11) and Wechser (6) reported new cases of EGPA in asthmatic patients during therapy with Omalizumab treatment. The latter could be explained by a reduction of immunosuppressive treatment that masked a previous EGPA, as occurred in anti-leukotriene therapeutic trials (9). We had a good clinical response because our patient continued steroid therapy, systemic and inhaled, at very low dosage (prednisone 5 mg/day); furthermore she was no

more admitted for more than five years despite an unchanged FEV1, probably due to an advanced bronchial remodeling. On the biological point of view, the introduction of Omalizumab leaded to the normalization of circulating eosinophils and p-ANCA, a fact that was more evident than serum IgE reduction, already induced and maintained by steroids. The effectiveness of Omalizumab on circulating eosinophils seems to be due to an increased apoptosis together with a reduction of IL-2 and IL-13 and GM-CSF production by lymphocytes (8). This pleiotrophism is probably the reason for the substantial anti-inflammatory effect of Omalizumab. Other clinical observations or clinical trials could better explain why some patients showed a good response, while others didn't.

References

1. Churg J, Strauss L. Allergic granulomatosis, allergic angiitis and periarteritis nodosa. Am J Pathol. 1951;27:277-301.
2. Szczeklik W, Jakiela R, Adamek D, et al. Cutting Edge issues in the Churg Strauss syndrome. Clin Rev Allergy Immunol. 2013;44:39-50.
3. Eustace JA, Nadasdy T, Choi M. Churg Strauss Disease. J Am Soc Nephrol. 1999;10:2048-55.
4. Szczeclic W, Sokolowska BM, Zuk J, Mastalerz L, et al. The course of asthma in Churg-Strauss Syndrome. J Asthma. 2011;48(2):183-7.
5. Cottin V, Khouatra C, Dubost R, Glerant JC, Cordier JE. Persistent airflow obstruction in asthma of patients with Churg-Strauss syndrome and long-term follow-up. Allergy. 2009;64(4):589-95.
6. Wechsler ME, Wong D, Miller MK, Miyasaki LL. Churg Strauss Syndrome in patients treated with Omalizumab. Chest ME Wechser. 2009;136:507-18.
7. Giavina-Bianchi P, Agondi R, Kalil J. One year administration of anti-IgE to a patient with Churg-Strauss-Syndrome. Int Arch Allergy Immunology. 2008;146:176-8.
8. Noga O et al. Effect of omalizumab treatment on peripheral eosinophil and T-lymphocyte function in patients with allergic asthma. J Allergy Clin Immunol. 2006;117:1493-9.
9. McDanel DL, Muller BA. The linkage between Churg-Strauss syndrome and leukotriene receptor antagonists: fact or fiction? Therapeutics and Clinical Risk Management. 2005:1(2);125-40.
10. Bargagli E, Rottoli P. Omalizumab treatment associated with Churg-strauss vasculitis. Int Arch Allergy Immunol. 2007;145:268.
11. Ruppert AM et al. J. Allergy Clin. Immunol. 2008;121(1):253-4.

Eur Ann Allergy Clin Immunol Vol 46, N 6, 229-231, 2014

P.V. Kolkhir[1], D.S. Pogorelov[2], T.P. Nekrasova[3], E.A. Nikitin[4], O.Y. Olisova[1], A.M. Kovrigina[4], A.L. Melikyan[4], L.R. Plieva[1], S.V. Moiseev[5], P.I. Novikov[5]

Hyperimmunoglobulinemia E and efficacy of elimination diet in two patients with Schnitzler syndrome

[1]I.M. Sechenov First Moscow State Medical University, Department of Dermatology and Venereology, Moscow, Russia
[2]Rostov State Medical University, Department of Dermatology and Venereology, Rostov-on-Don, Russia
[3]I.M. Sechenov First Moscow State Medical University, Department of Pathology, Moscow, Russia
[4]Russian Research Center for Hematology, Out-patient Department, Moscow, Russia
[5]I.M. Sechenov First Moscow State Medical University, Department of Rheumatology, Moscow, Russia

Key words
Schnitzler syndrome; chronic urticaria; IgE; diet; hyperimmunoglobulinemia E

Corresponding author
Pavel V. Kolkhir
Varshavskoe shosse, 152, b.7, 317
Moscow 117405, Russian Federation
Email: arthate@yandex.ru
Phone: +7 916 5030 455

Summary
Background. *Schnitzler syndrome (SS) is a rare clinical entity characterized by chronic recurrent urticarial rash, monoclonal IgM gammopathy, intermittent fever and other symptoms. In this report, we present the cases of two patients with SS: a male and a female aged 50 and 49 years, respectively. Both patients had hyperimmunoglobulinemia E and showed good response to elimination diet.* ***Methods.*** *The patients had chronic urticaria, IgM gammopathy and an elevation of the serum levels of inflammation markers. Total IgE levels were found to be high (2000 U/ml and 540 U/ml, respectively). No underlying causes for hyperimmunoglobulinemia E (allergy, parasites, etc.) were revealed. The first patient did not respond to the treatment with antihistamines, while the second one responded only to high doses. The response to prednisolone in the second patient was incomplete.* ***Results.*** *Following a strict elimination diet resulted in marked improvement in skin lesions in both patients. In one of our patients we observed a decrease in IgE and IgM levels after a 3 week diet. The systemic symptoms persisted and improved only after adding pefloxacin, followed by a 3-day empirical course of intravenous prednisone in the first patient and a course of plasmapheresis in the second one.* ***Conclusion.*** *The high serum levels of total IgE may be associated with chronic urticaria activity, severe disease course and a poor response to treatment with antihistamines, and may be considered a possible marker of a subset of patients with SS showing a good response to the restriction diet. In general, we can assume that elimination diet can have an influence on the skin lesions and other symptoms of SS as well as on total IgE and IgM levels, but such association, the underlying mechanisms and the reasons for excessive IgE synthesis should be investigated in further studies.*

Introduction

Schnitzler syndrome is a rare clinical entity which associates urticarial rash, enlarged lymph nodes, bone pain, fever, arthralgia or arthritis, hepato- or splenomegaly, leukocytosis, elevated erythrocyte sedimentation rate (ESR), and a monoclonal IgM gammopathy (1).

Case 1

A Russian 50 year-old male was diagnosed with SS in February 2013. Since the end of 2007, he complained of itchy urticarial rash (**figure 1 a**, **b**), general malaise, abdominal pain, bone pain in extremities, sweating, and fever. He had enlarged lymph nodes. High serum levels of total IgE persisted from 2011 till 2013,

Figure 1 a, b *- Multiple urticarial lesions on trunk of the first patient with SS.*

Figure 1a

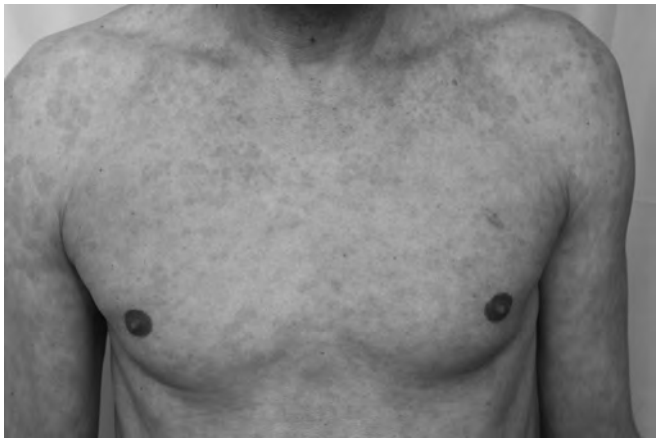
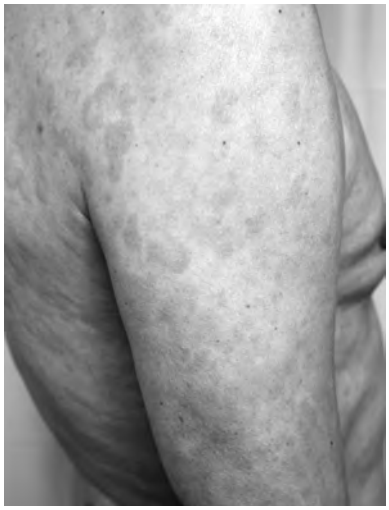
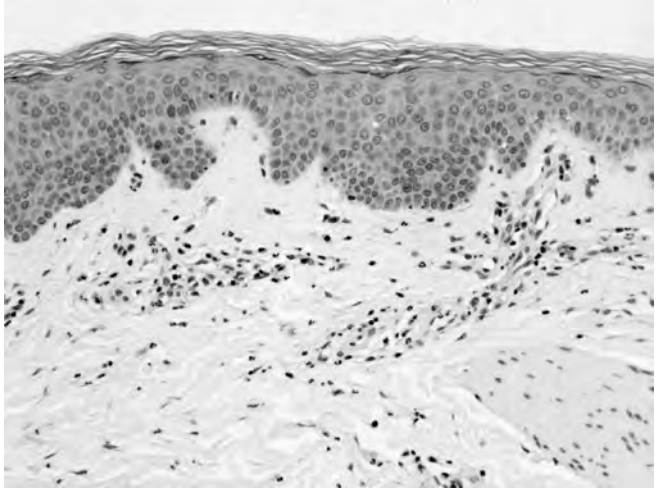


Figure 1 b



with an average level of 2000 U/ml. The level of specific IgE to different allergens was within normal limits, and the patient had no history of allergy. Laboratory analyses showed leukocytosis, accelerated ESR (25 mm/h), increased levels of CRP (25 mg/l), total IgM (5.1 g/l), total IgA (277 U/ml), rheumatoid factor (436 U/ml) and fibrinogen (7.85 g/l). IgM-κ paraprotein (5.7 g/l) was found. Tests for cryoglobulins and autoantibodies were negative. A skin biopsy was performed (**figure 2**). A bone marrow biopsy revealed no specific involvement of the bone marrow. The patient was initially treated with antihistamines and antibiotics, without any positive effect. His past medical history was remarkable for the significant improvement in skin symptoms when he was put on elimination diet. In June 2013, we administered him a 3-week course of elimination diet accompanied by pefloxacin (800 mg/d), followed by a 3-day empirical course of intravenous prednisone (500 mg/d), that resulted in a nearly complete resolution of cutaneous symptoms, a significant decrease in bone pain, and disappearance of fever. Interestingly, total IgE and IgM levels decreased at the end of the 3-week diet (from 2156 to 500 U/ml and 5.1 to 3.56 g/l, respectively).

Figure 2 *- Hyperkeratosis, irregular acanthosis. Loose lymphocytic perivascular infiltrate with admixture of plasma cells, macrophages, neutrophils in the upper dermis. Morphological signs of neutrophilic dermatosis (hematoxylin and eosin X100).*



Case 2

A Russian 49 year-old female was diagnosed with SS in November 2013. Since the end of 2000, she complained of itchy urticarial rash (**figure 3**), swelling of lips, eyelids, throat and pain in the small joints of hands and feet. The last exacerbation of the disease began in April 2013. The urticarial lesions occurred daily after exposure to pressure (from shoulder strap of bags, tight-fitting shoes, etc.) and the symptoms of angioedema worsened. Since 2002, the patient had high serum levels of total IgE (540 U/ml in 2013) and IgM (10.6 g/l in 2013). The patient had no history of allergy. Laboratory analyses showed increased serum levels of rheumatoid factor (27.7 U/ml), D-dimer (3.36 μg/ml), CRP (18.4 mg/l), fibrinogen (6.7 g/l), beta-2 microglobulin (3.16 mg/l), IgM-κ paraprotein (4 g/l). A bone marrow biopsy enabled to rule out multiple myeloma. A skin biopsy was performed (**figure 4**).

Figure 3 *- Isolated urticarial rash on left shoulder in the second case.*

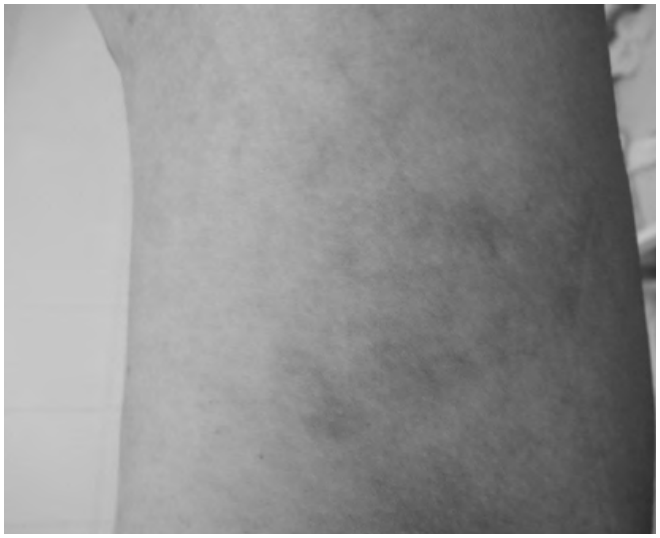
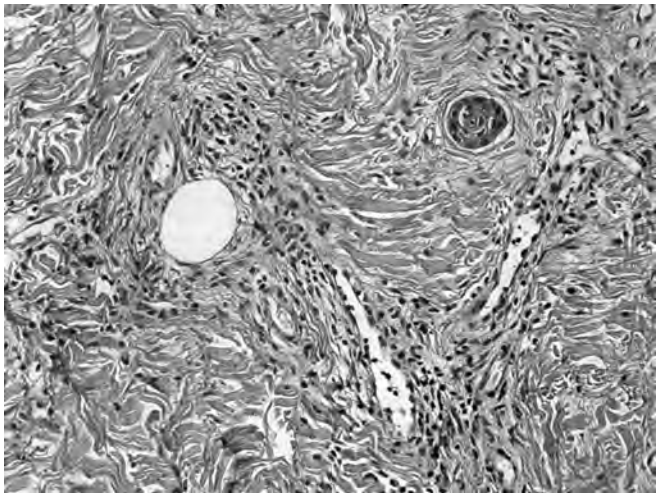


Figure 4 *- Perivascular infiltrates consisting of lymphocytes, macrophages, plasma and mast cells in the dermis. Sclerosis of dermal collagen fibers. Morphological signs of chronic dermatitis (hematoxylin and eosin X100).*



The response to high-dose antihistamines was initially good, but became insufficient. Prednisolone and dexamethasone intramuscular injections had only temporary effects. As in the first case, following strict elimination diet resulted in an almost complete resolution of cutaneous symptoms and in a considerable decrease in bone pain. The patient took several courses of plasmapheresis with positive effect.

In this patient, IgE and IgM serum levels fluctuated, regardless of the diet and the therapy.

Discussion

In our study we used a 3-week elimination diet suggested by Metz M. and Magerl M. (2). Patients could only take bread without any additives, potatoes, rise, raw cereals, wheat pasta (made without eggs), butter, vegetable oil, fresh milk, cream without food stabilizers, natural yoghurt, cottage cheese, some Gouda cheese, fresh meat without spices, any vegetables, salt, onion, spring onion, mineral water, coffee, black and green tea (without flavorings). In both patients, the foods were excluded on the basis of the presence of pseudoallergens like biogenic amines and food additives (preservatives, colorings, etc.). We initially assumed that it is pseudoallergen-low diet that might have helped to reduce the urticarial activity (2). But having conducted a 3-week diet, we observed a considerable decrease in total IgE level in the first case. We found this phenomenon quite interesting. It is unknown if such effects of elimination diet on urticarial activity are based on allergic, non-allergic, mixed mechanisms (3) or associated with altered gastroduodenal permeability (4).

In both our patients, IgE levels were significantly elevated without any underlying reasons (allergy, parasites etc). To our knowledge, the occurrence of IgE hyperimmunoglobulinemia and the efficacy of elimination diet in SS has not been described in literature so far. The high serum level of total IgE may be associated with chronic urticaria activity, severe disease course and a poor response to treatment with antihistamines (5) and may be considered a possible marker of a subset of patients with SS showing a good response to the restriction diet. As we have mentioned before, in one of our patients we observed a decrease in IgE and IgM levels after a 3-week diet. But it is still difficult to say whether it was a spontaneous decrease or if this decline was associated with the diet, pharmacotherapy or both.

In general, we can assume that elimination diet can have an influence on the skin lesions and other symptoms of SS (like bone pain in the second patient) as well as on total IgE and IgM levels, but such association and the underlying mechanisms require further investigation.

References

1. Simon A, Asli B, Braun-Falco M et al. Schnitzler's syndrome: diagnosis, treatment, and follow-up. Allergy. 2013;68(5):562-8.
2. Metz M, Magerl M. Urticaria and Angioedema. Chapter 7. Eds: T. Zuberbier, C. Grattan, M. Maurer. Springer International edition. 2010;144-6.
3. Wedi B, Kapp A. Current position of the role of allergic and non-allergic food hypersensitivity in urticaria. Hautarzt. 2006;57:101-7.
4. Buhner S, Reese I, Kuehl F et al. Pseudoallergic reactions in chronic urticaria are associated with altered gastroduodenal permeability. Allergy. 2004;59:1118-23.
5. Kessel A, Helou W, Bamberger E et al. Elevated serum total IgE - a potential marker for severe chronic urticaria. Int Arch Allergy Immunol. 2010;153(3):288-93.

Eur Ann Allergy Clin Immunol Vol 46, N 6, 232-233, 2014

S. Voltolini, S. Pellegrini, M. Contatore, D. Bignardi, P. Minale

New risks from ancient food dyes: cochineal red allergy

Allergy Department, IRCCS Azienda Ospedaliera Universitaria San Martino IST, Genova, Italy

Key words
Carmine red; Cochineal red; anaphylaxis; food additives; dyes

Summary
This study reports an unusual case of IgE-mediated hypersensitivity to Cochineal red or Carmine red, a coloring agent of natural origin. Although the risk of anaphylactic reactions is well known, since the nineties the use of this additive seems to be nowadays on the rise. The problem of labeling of additives used in handmade food products is highlighted.

Corresponding author
Susanna Voltolini
E-mail: susanna.voltolini@hsanmartino.it

Introduction

Among food and drug additives, Carmine or Cochineal Red is one of the best known as a cause of allergic reactions (1).
This natural red dye derives from the bodies of female *Dactylopius coccus*, insects that grow on cochineal cacti widespread in Central and Southern America, Southern Europe, and India. This dye has been widely used since sixteenth century as a coloring agent in processed food and drinks, cosmetics and textiles. Nowadays, it is found in hamburgers, sausages, alcoholic and non-alcoholic drinks, sweets and fruit yoghurts. However, its identification in such a various range of products is difficult, particularly in fresh processed food such as those handmade by butchers.
During the past 20 years, several Authors reported about severe allergic reactions following the ingestion of carmine in foods and drinks (2,3). Moreover, allergic rhinitis and asthma in subjects exposed to powdered carmine dye in occupational settings is well known (4,5). IgE-mediated food and respiratory hypersensitivity has been shown (6), and cheilitis following contact with lipsticks colored with carmine has been reported as well (7). Some Authors hypothesize that the presence of the dye in cosmetics could be the reason for the female prevalence of this sort of allergy.
The first identification of allergens of cochineal red was reported by Quirce et al. in 1994 (4). More recently, Japanese researchers characterized and cloned a 38 kDa phospholipase or related enzyme, homologous to other similar proteins in insects, as the major allergen (8).

Case report

A 32 years old woman with no prior history of allergies was referred in 2011 to our clinic for an episode of generalized ur-

ticaria, associated with eyelid oedema and rhinitis occurring immediately after the intake of a protein-vitamin supplement (Car-go; Recordati, Italy). During the previous 5 years she experienced some other similar but less severe episodes after eating sausages or spicy meat (kebab). The label on the vitamin supplement reported Carmine (in addition to amino acids, vitamins of group B, C and E), which led us to suspect a reaction to the dye. Notably, carmine red has been recently removed from the drug.
A SPTt with the integrator, performed diluting the powder content of one capsule (0.1 mg/ml NaCl) was performed and scored positive, eliciting erythema and a pruritic 5 mm wheal. The same SPT scored negative in five control subjects. Skin tests with inhalant and food allergens, including various kinds of meat and spices, were negative. Specific IgE to red cochineal (ImmunoCAP F340) was 0.19 KU/l. ImmunoCAP for the common foods scored negative. The basophil activation test (Flow CAST from Bühlmann Laboratories, Schönenbuch, Switzerland) to Mix dyes 1 and 2 (including E120 and E124) and with the protein-vitamin supplement "Car-go" was negative.
Some months after this episode, the patient experienced a new, more severe, reaction with angioedema of lips and face and slight laryngeal involvement, some hours after ingestion of a handmade turkey hamburger. The butcher confirmed the use of some carmine-colored sausages for the preparation of the hamburger.

Discussion

This case confirms the actual risk of allergic reactions induced by Carmine red present not only in food, but also in dietary supplements and OTC drugs. Its increased use may expose the population to a relevant risk of IgE sensitization. The possible occurrence of symptoms some hours after the ingestion of the dye is reported in the literature, and may result in undiagnosed cases labelled as "idiopathic anaphylaxis".
The natural Cochineal red, classified in Europe as E120, must be distinguished from the synthetic form, the Cochineal Red A also named Ponceau 4R (E124), belonging to the azo-dyes. It is known as histamine-liberator, but not as IgE sensitizing agent.
We believe that accurate, more specific and strict regulation on labelling (both at an international and national level) is crucial in safeguarding health.
Only recently the FDA ruled that food, drugs and cosmetic products containing coloured additives like cochineal extracts and carminic acid must be clearly labelled. In Europe, the regulation on food labelling includes additives and dyes: red dyes are listed from E120 to E129 (**table 1**). The system appears to be efficient at level of industrial products, while handmade foods are more at risk of undeclared manipulation. Further, the existence of two similar Cochineal Red dyes, E120 and E124, may represent a risk of mistake in most of European countries. Notably, the synthetic, non- allergenic one (E124) is not allowed in Norway and Finland, and also in USA because of a supposed carcinogenic risk.
The potential risk of allergic reactions due to natural cochineal red would deserve greater attention among specialists. The demonstration of the mechanism needs more sensitive diagnostic methods. Although very low, the IgE specific level (0,19 KU/l) obtained in the study can be considered positive, because in the last years the use of the whole quantitative scale of antibodies presence is preferred, rather than the cut-off value of 0,35 KU/l in the CAP-system. A diagnostic extract for skin test is not available.
The identification of the major allergen and the use of recombinant and molecular diagnosis could be of great importance to ameliorate the diagnosis in a next future.

Table 1*- Red colouring additives (EU nomenclature).*

E 120	Cochineal, Carminic acid, Carmines
E 122	Azorubine, Carmoisine
E 123	Amaranth
E 124	Ponceau 4 R, Cochineal Red A
E 127	Erythrosine
E 129	Allura Red AC

References

1. Greenhawt MJ, Baldwin JL. Carmine dye and cochineal extract: hidden allergens no more. Ann Allergy Asthma Immunol. 2009 Jul;103(1):73-5.
2. Beaudouin E, Kanny G, Lambert H, Fremont S, Moneret-Vautrin DA. Food anaphylaxis following ingestion of carmine. Ann Allergy Asthma Immunol. 1995May;74(5):427-30.
3. Wüthrich B, Kägi MK, Stücker W. Anaphylactic reactions to ingested carmine (E120). Allergy. 1997;52(11):1133-7.
4. Quirce S, Cuevas M, Olaguibel JM, Tabar AI. Occupational asthma and immunologic responses induced by inhaled carmine among employees at a factory making natural dyes. J Allergy Clin Immunol. 1994;93(1Pt1):44-52.
5. Ferrer A, Marco FM, Andreu C, Sempere JM. Occupational asthma to carmine in a butcher. Int Arch Allergy Immunol. 2005;138(3):243-50.
6. Acero S, Tabar AI, Alvarez MJ, Garcia BE, Olaguibel JM, Moneo I. Occupational asthma and food allergy due to carmine. Allergy. 1998;53(9):897-901.
7. Sarkany I, Meara Rh, Everall J. Cheilitis due to carmine in lip salve. Trans St Johns Hosp Dermatol Soc. 1961;46:39-40
8. Ohgiya Y, Arakawa F, Akiyama H, Yoshioka Y, Hayashi Y, Sakai S, Ito S, Yamakawa Y, Ohgiya S, Ikezawa Z, Teshima R. Molecular cloning, expression, and characterization of a major 38-kd cochineal allergen. J Allergy Clin Immunol. 2009;123(5):1157-62.
9. Commission Regulation (EU) No 231/2012 of 9 March 2012. Official Journal of the European Union 22.3.2012.

M. Gelardi

"Overlapped" rhinitis: a real trap for rhinoallergologists

Key words
Vasomotor rhinitis; allergic rhinitis; nasal cytology; therapy rhinitis

Summary
Under the broad heading of "vasomotor" rhinitis two big groups can be distinguished: allergic rhinitis (IgE-mediated), and nonallergic rhinitis. Since they are two separate nosological entities, they can co-exist in the same patient, classifying themselves in the group of "overlapped" rhinitis (OR). Although not absolutely rare (indeed it is estimated a 15-20% incidence among all vasomotor rhinopathies), this condition is not investigated and diagnosed, with significant implications in the clinical-diagnostic and therapeutic field.

Matteo Gelardi
Section of Otolaryngology
Department of Basic Medical Science,
Neuroscience and Sensory Organs
University of Bari, Italy
E-mail: gelardim@inwind.it

Under the broad heading of "vasomotor" rhinitis, two big groups can be distinguished: allergic rhinitis (IgE-mediated) (1), and nonallergic rhinitis, better defined as "cellular" rhinitis, represented by NARES (non-allergic rhinitis with eosinophils), NARMA (non-allergic rhinitis with mast cells), and NARES-MA (non-allergic rhinitis with eosinophils and mast cells) (2-4), whose etiology is unknown to date. Since they are two separate nosological entities, they can co-exist in the same patient, as well as for other diseases (diabetes, arterial hypertension, etc.), classifying themselves in the group of "overlapped" rhinitis (OR) (5). Although not absolutely rare (indeed it is estimated a 15-20% incidence among all vasomotor rhinopathies), this condition is not investigated and diagnosed, with significant implications in the clinical-diagnostic and therapeutic field.

On clinical level, the patient with OR, if compared to the typical patient sensitized to house dust mite, shows a more intense (sneezing, rhinorrhea, nasal itching with bouts of sneezing) and persistent symptomatology. Indeed, in patients sensitized to house dust mite, symptoms are most often characterized by a "moderate" intensity. In the case of OR with sensitization to "persistent" pollens, such as gramineae and parietaria, symptoms remain even in the months when the presence of airborne pollen particles is almost absent.

The diagnosis of these clinical conditions requires: an accurate and in-depth anamnesis; a skin prick test correlated to the anamnestic history and to the pollen calendar of the area where the patient lives; a nasal fibre-endoscopy; and, finally, a nasal cytology (6,7).

By means of allergologic diagnostics, all the common "environmental" allergens should be tested in addition to those correlated to the patient's type of job, hobby, etc.

The endoscopic exam of nasal cavities will evaluate the characteristics of mucosa (edema, hyperemia, presence of secretions), and exclude anatomic alterations (septal deviations and perfora-

Table 1 *- When to suspect "overlapping" of different rhinopathies (allergic rhinitis + NARESMA, NARES or NARMA).*

Clinical criteria
• Chronic "vasomotor" rhinitis symptoms (nasal congestion, rhinorrhea, sneezing a salve), present even outside the pollen season, in a patient with positive skin prick test and/or RAST test • Increased "vasomotor"-type nasal reactivity to non specific stimuli (sudden changes in temperature, light stimuli, strong smells, cigarette smoke, exposure to chlorine (swimming), etc.) • Disturbances of taste and smell (suspect onset of nasal polyposis) • Positive family history of nasal polyposis, NARES, NARMA, NARESMA, asthma, sensitivity to acetylsalicylic acid, hypo-anosmia, vasomotor rhinitis labeled "non specific", previous turbinate surgery for nasal congestion which gave poor medium- to long-term results • Recurrent use of nasal decongestants • Little or no clinical benefit following turbinate surgery for nasal congestion • Little or no clinical benefit following a cycle of specific immunotherapy (SIT)
Cytologic criteria
• In the forms with "persistent" symptoms, overlapping should be suspected in all patients with a rhinocytogram showing a cell profile different from that associated with "persistent minimal inflammation" (i.e. different from that characterised by numerous neutrophils, some lymphocytes and occasional eosinophils, with rare signs of degranulation), where there are eosinophils > 20% and/or mast cells > 10%. • In the forms with "intermittent" symptoms, overlapping should be suspected in all patients with a positive rhinocytogram (eosinophils > 20% and/or mast cells > 10%) outside the pollen season for the allergen/s identified by allergy testing (skin prick test and/or RAST test).
In rhinocytology, November tends to be preferred for "unraveling" overlapping rhinopathies, as this is the month in which most airborne pollens are absent. The presence of immuno-inflammatory cells (eosinophils and/or mast cells) associated with rhinitis symptoms confirms the presence of overlapping diseases.

RAST, radio-allergo-sorbent test

tions) and hyperplastic-granulomatous (nasal polyposis, antrochoanal polyp, etc.) or infectious (rhinosinusitis) diseases.

Nasal cytology, by researching eosinophils, mast cells, neutrophils, lymphocytes, bacteria, and fungal spores correlated to patient's clinical history, allergy tests and endoscopy, will help to "unravel" the forms of OR (**table 1**).

Against this background, doctors could find themselves facing a set of clinical conditions which could be partly summarized in the following clinical cases:

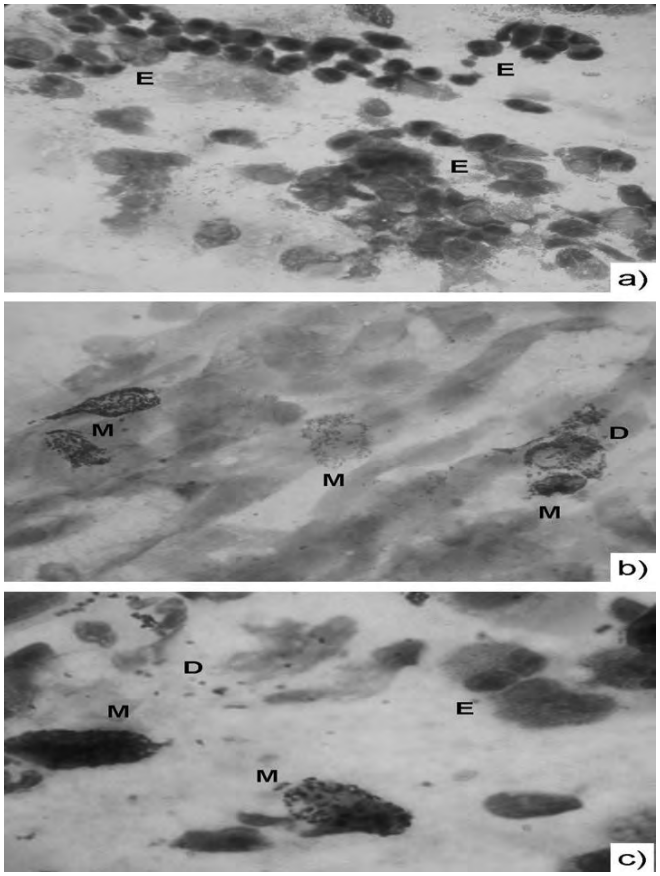
a) allergy to house dust mite associated to NARES. The patient will show an intense and persistent symptomatology, with a cytological framework dominated by a high number of eosinophils (> 20%), degranulated in the most part (**figure 1a**), contrary to "pure" allergic rhinitis to house dust mite only, characterized by a large number of neutrophils, occasional eosinophils, and almost no degranulation;

b) allergy to cypress, associated to NARMA. The patient will show symptoms no longer limited to the first 3-4 months of the year, but persistently, with a cytological framework represented by numerous mast cells, degranulated in the most part, even outside the correspondent pollen season (**figure 1b**);

c) allergy to gramineae and parietaria associated to NARESMA. The patient will show a rhinological symptomatology no longer limited to correspondent pollen periods (April-October), but persistently, with a cytological framework dominated by numerous eosinophils and mast cells, degranulated in the most part, even outside correspondent pollen seasons (**figure 1c**).

From a purely therapeutic perspective, ORs are real "traps" for the rhinoallergologist. Within the field of allergology, indeed, the patient with OR, when it is not diagnosed, will be subjected to Allergen-specific Immunotherapy (SIT). Besides the real and proven therapeutic effects of SIT, as reported by international literature (9), the patient will have poor clinical outcome from this therapy. While acting on the IgE-mediated rhinopathy, the SIT will have no therapeutic action on the associated non-IgE-mediated vasomotor component, generating dissatisfaction at the end of the treatment. The same problem will affect the patient subjected to surgical treatment of the turbinates, regardless of

Figure 1a, b, c *- "Overlapped" rhinitis. a) Allergic rhinitis to house dust mite associated to NARES (cytological sampling performed during the summer period under conditions when house dust mite concentration is reduced). There is a large number of partially degranulated eosinophils (E); b) allergic rhinitis to cypress associated to NARMA (sampling performed in November, in the absence of cypress pollens). There is a large number of partially degranulated mast cells (M); c) allergic rhinitis to gramineae and parietaria associated to NARESMA (nasal cytological sampling performed in November, outside the pollen period). There is a large number of partially degranulated eosinophils and mast cells. MGG staining, x 1,000.*



the type of surgical procedure used (laser, radiofrequencies, submucous resection of the turbinate, etc.), whose benefits will be limited in time as well. We do believe that these conditions are the expression of some syndromes nowadays classified as Severe Chronic Upper Respiratory Disease (SCUAD) (10). Therefore, reaching a precise diagnosis is always necessary, in order to inform the patient of his/her clinical condition, since we are more convinced that "diagnosis is always helpful for the therapy".

When treating the ORs, the SIT has the same indications as in the "pure" IgE-mediated forms, and therefore the therapy will be advised to the patient who will be informed in advance that, even after terminating the SIT, s/he will have to continue the medical treatment (corticosteroid and topical and/or systemic antihistamine). Likewise, a program of SIT and cycles of corticosteroid and topical and/or systemic antihistamine therapy will be recommended, right after the post-surgery recovery period, even to those patients who undertake surgical treatment of the turbinates, in order to monitor rhinitis symptomatology and prevent relapses.

In the light of the above, it is desirable that the diagnosis of rhino-allergy increasingly relies on investigations such as rhinoendoscopy and nasal cytology, still considered today as "second level" (1), which are necessary in order to "unravel" the ORs, clinical conditions still too often little known and, therefore, not diagnosed.

References

1. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, van Weel C, et al.World Health Organization; GA(2)LEN; AllerGen. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update (in collaboration with the World Health Organization, GA2LEN and AllerGen). Allergy. 2008;63(Suppl.86):8-160.
2. Jacobs RL, Freedman PM, Boswell RN: Non allergic rhinitis with eosinophilia (NARES syndrome): clinical and immunologic presentation. J Allergy Clin Immunol. 1981;67:253-7.
3. Connell JT: Nasal mastocytosis. J Allergy. 1969;43:182-9.
4. Gelardi M, Maselli Del Giudice A, Fiorella ML, Fiorella R, Russo C, Soleti P, Di Gioacchino M, Ciprandi G. Non-allergic rhinitis with eosinophils and mast cells (NARESMA) constitutes a new severe nasal disorder. Int J Immunopathol Pharmacol. 2008;23:325-31.
5. Gelardi M, Russo C, Fiorella ML, Fiorella R, Canonica GW, Passalacqua G. When allergic rhinitis is not only allergic. Am J Rhinol Allergy. 2009;23(3):312-5.
6. Bousquet J, Heinzerling L, Bachert C, Papadopoulos NG, Bousquet PJ, Burney PG, Canonica GW, and Global Allergy and Asthma European Network; Allergic Rhinitis and its Impact on Asthma. Practical guide to skin prick tests in allergy to aeroallergens. Allergy. 2012;67(1):18-24.
7. Gelardi M. Atlas of nasal cytology. Edi Ermes Milan, Italy. 2012.
8. Gelardi M, Incorvaia C, Passalacqua G, Quaranta N, Frati F. The classification of allergic rhinitis and its cytological correlate. Allergy. 2011;66:1624-5.
9. Hankin CS, Cox L, Bronstone A,Wang Z. Allergy immunotherapy: reduced health care costs in adults and children with allergic rhinitis. J Allergy Clin Immunol. 2013;131(4):1084-91.
10. Bousquet J, Bachert C, Canonica GW, Casale TB, Cruz AA, Lockey RJ, Zuberbier T; Extended Global Allergy and Asthma European Network, World Allergy Organization and Allergic Rhinitis and its Impact on Asthma Study Group. Unmet needs in severe chronic upper airway disease (SCUAD). J Allergy Clin Immunol. 2009;124(3):428-33.

O. Quercia[1], F. Emiliani[1], B. Stefanini[2], G.F. Stefanini[3]

The importance of educating subjects entitled to use an adrenaline auto-injector for self-administration

[1]Unità ad Alta Specializzazione di Allergologia, Ospedale di Faenza, (RA), Italy
Phone: +39 0546 60 11 65, fax: +39 0546 60 10 68, e-mail: fa.allergologia@ausl.ra.it
[2]Medical student, E-mail: gf.stefanini@ausl.ra.it
[3]Dpt of Internal Medicine, Ospedale di Faenza, (RA), Italy

Key words
Anaphylaxis; adrenaline; compliance to treatment

Summary
An educational program, consisting in a clear explanation of the technical aspects and use of the adrenaline auto-injection devices (AAD) and in a practical test utilizing a demonstration kit was given to 350 patients from our outpatient clinic. AAD was also distributed to 50 patients formerly followed by another allergy clinic without training. At practical control test, only 10% of the untrained patients where able to correctly use the AAD versus 80% of trained subjects. Since AAD is a life-saving procedure in severe anaphylactic episodes, this attempt to improve the ability of the patient to comply with the procedure can improve the efficacy of the treatment and eventually the patient's health.

Corresponding author
Tel: +39 0546 60 19 73
E-mail: gf.stefanini@ausl.ra.it

Following a treatment strictly has always represented a problem in medicine. A gap in the compliance of the treatment in chronic illnesses could lead on the one hand to several problems for patients to face, and on the other hand it could spoil medical resources, but more importantly in emergency cases it could become life threatening.

The World Allergy Organization Guidelines for the Assessment and Management of Anaphylaxis stated in a recent revision (1) that in spite of the lack of controlled clinical trials, the first therapeutic attempt should be adrenaline intramuscular injections (2). This procedure can stop the progression of the anaphylactic shock (3), that could in a matter of minutes lead to death especially in more fragile subjects, such as elderly patients, pregnant women, newborns or patients affected by cardiovascular comorbidity or following beta-blockers therapies (4). The life-saving role of adrenaline administration makes it a milestone of treatment of severe acute allergic reactions, and led the Italian national drug regulatory organism to include the adrenaline auto-injection devices (AAD) for self-administration in the free of charge therapeutic tools for allergologist selected patients. Moreover, the evaluation strategy formerly confined to symptom driven selection, has been extended to an evaluation of the specific antigen intrinsic hazard (5) increasing the AAD prescriptions.

This is why we have implemented a patient-information-program for those eligible for adrenaline auto-injection devices (AAD) for self-administration (6). The main goal of the program is to teach them how to face the two main obstacles in adrenalin auto-injections: first of all the complications directly connected to the use of the AAD device (7), and then the accuracy in detecting the auto-injection proper correct time. Moreover, it is worth mentioning that a correct information reduces the risk of an accidental injection (8).

Our educational program consists in a clear explanation of the technical aspects and use of the AAD device with particular

emphasis on the timing, followed by a practical test utilizing a demonstration kit that is given to the patient along with the AAD device, test which is repeated annually.

In the last two years, in our outpatients clinic after a careful evaluation we selected 350 patients to receive the AAD device; an educational program together with an ample encouragement and a strengthening of motivations was provided to each selected patient; moreover, in the last year we have also admitted to our outpatient clinic 50 patients formerly followed by another allergy clinic from a nearby town where, after a scrupulous selection, the AAD device was given without a formal information program leaving the patients to deduce such information from the written instructions contained in the AAD device package.

All these newcomers underwent a practical test with the demonstration kit, and we observed that only 10% where actually able to use the AAD device correctly versus 80% of our patients to whom we had offered the direct verbal educational trial.

Moreover, only 25% of our historical patients versus 68% of the newcomers were not aware of the expiration date of the device, leaving them to face consequent risks of using a spoiled and ineffective drug with potentially lethal consequences, a data which was reported as high as 54% in other studies (9).

The main obstacles we observed were on the one hand the incapability of the patients to accept the gravity and possible lethal harm of the disease itself, and on the other hand the fear of the procedure and its potential side effects.

These aspects are in line with previous observations reporting that besides the technical difficulties due to different auto-injection devices, complex emotional and behavioural factors from patients and their relatives come into play and do play a crucial role (10,11).

In order to optimize the result we believe that the AAD prescription must be accompanied by a complete and comprehensive information, and proper motivation; the yearly proposal of the educational test adds an useful feedback for the doctor to a boost of patient's knowledge. This observation could be useful to all allergists who have to evaluate and give AAD devices, and as far as we are concerned it has prompted us to substantiate the educational program on the topic with the attempt to improve the ability of the patient to comply with the procedure and strengthen the efficacy of our treatments in such a delicate matter.

References

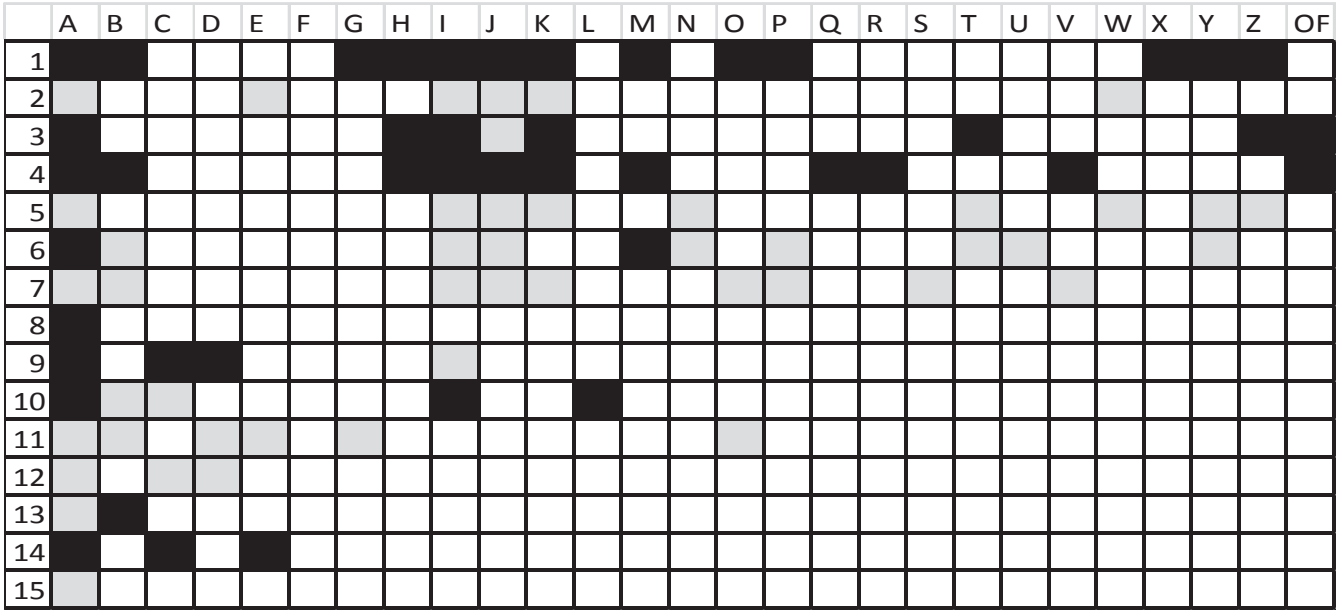
1. Simons FE, Ardusso LR, Dimov V, Ebisawa M, El-Gamal YM, Lockey RF, Sanchez-Borges M, Senna GE, Sheikh A, Thong BY, Worm M. World allergy organization anaphylaxis guidelines: 2013 update of the evidence base. Int Arch Allergy Immunol. 2013;162(3):193-204.
2. Lieberman PL. Recognition and first-line treatment of anaphylaxis. Am J Med. 2014Jan;127(1 Suppl):S6-S11.
3. Sheikh A, Shehata YA, Brown SG, Simons FE. Adrenaline for the treatment of anaphylaxis: cochrane systematic review. Allergy. 2009Feb;64(2):204-12.
4. Kemp SF, Lockey RF, Simons FE; World Allergy Organization ad hoc Committee on Epinephrine in Anaphylaxis. Epinephrine: the drug of choice for anaphylaxis. A statement of the World Allergy Organization. Allergy. 2008Aug;63(8):1061-70.
5. Asero R, Antonicelli L, Arena A, Bommarito L, Caruso B, Colombo G, Crivellaro M, De Carli M, Della Torre E, Della Torre F, Heffler E, Lodi Rizzini F, Longo R, Manzotti G, Marcotulli M, Melchiorre A, Minale P, Morandi P, Moreni B, Moschella A, Murzilli F, Nebiolo F, Poppa M, Randazzos S, Rossi G, Senna GE. Epinephrine autoinjector prescription in food-allergic adults: symptom-based only or allergen-based also? An Italian multi-centre study. Eur Ann Allergy Clin Immunol. 2010 Feb;42(1):25-31.
6. Gupta R.S. Anaphylaxis in the young adult population. Am J Med. 2014Jan;127(1Suppl):S17-24.
7. Frew A.J. What are the 'ideal' features of an adrenaline (epinephrine) auto-injector in the treatment of anaphylaxis? Allergy. 2011Jan;66(1):15-24.
8. Simons KJ, Simons F.E. Epinephrine and its use in anaphylaxis: current issues. Curr Opin Allergy Clin Immunol. 2010Aug;10(4):354-61.
9. Fischer J, Knaudt B, Caroli U.M, Biedermann T. Factory packed and expired - about emergency insect sting kits. J DtschDermatolGes. 2008Sep;6(9):729-33.
10. Gallagher M, Worth A, Cunningham-Burley S, Sheikh A. Epinephrine auto-injector use in adolescents at risk of anaphylaxis: a qualitative study in Scotland, UK Clinical & Experimental Allergy. 2011;41,869-77.
11. Brown J, Tuthill D, Alfaham M, Spear E. A randomized maternal evaluation of epinephrine autoinjection devices. Pediatr Allergy Immunol. 2013Mar;24(2):173-7.

ERRATUM

In patients with LTP syndrome food-specific IgE show a predictable hierarchical order
R. Asero

In Volume 46, number 4, pp. 142-146, Table 2 was printed incorrectly. Please find the corrected Table 2 printed below.

Table 2 - *Clinically offending foods in patients with LTP hypersensitivity.*



A: Peach; B: Apple; C: Cherry; D: Apricot; E: Plum; F: Pear; G: Strawberry; H: Almond; I: Walnut; J: Hazelnut; K: Peanut; L: Maize; M: Rice; N: Lettuce; O: Kiwi; P: Tomato; Q: Lentil; R: Saffron; S: Melon; T:Pistachio; U: Onion; V: Rucola; W: Cashew; X: Pineapple; Y: Fennel; Z:Other nuts (Brazil nut, coconut, macadamia nut); OF: Other Foods (Bell pepper, Pitaya).

A black box indicates systemic symptoms following the ingestion of a food. A grey box indicates local symptoms (Oral allergy syndrome) and a white box indicates a tolerated food.

ANNOUNCEMENT

European Annals of Allergy and Clinical Immunology

The new online submission system

European Annals of Allergy and Clinical Immunology is proud to announce that a fully online submission and review system for all papers has been recently implemented.
Electronic submission will allow a more efficient processing of manuscripts and will offer authors the option to track the progress of the review process whenever they need to.
The link to this system has been made available on the new journal website, that has been launched simultaneously with the submission system at: *www.eurannallergyimm.com.*

We encouraged all Authors to submit manuscripts through our dedicated online peer review system; manuscripts will no longer be accepted for review by email or in paper form.
We advise all the authors to carefully read the instructions for authors before starting the submissions.
During submission, Authors will be first asked to select the article type, enter the manuscript title and provide author information. Through a menu, a general topic area should be selected: these will help us match manuscripts to the best available editors and reviewers.
Reviewers will access papers via the system website and will be directed to it when requested by email to evaluate submissions.
For full information on the manuscript preparation Authors are invited to read our updated Guide for Authors available on the Journal website.

Full Authors Guidelines, online Submission System link, Journal Publishing Agreement and Conflict of interest forms are available on new Journal website:
www.eurannallergyimm.com