Mattioli 1885 | Issn 1764-1489 | Volume 40 N. 4 /2008 - December 2008

European Annals of Allergy and Clinical Immunology

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

4/2008

Component-resolved diagnosis of plant food allergy by SPT

Suspected acute allergic reactions

Body mass index and airway hyper-responsiveness in individuals without respiratory disease

The nocebo effect during oral challenge in subjects with adverse drug reactions

Natural rubber latex allergy in children

A lipid extract of *Perna canaliculus* affects the expression of pro-inflammatory cytokines in a rat adjuvant-induced arthritis model

Poste Italiane s.p.a. - Sped. in A. P. - D.L. 353/2003 (conv. in L. 27/02/2004 n. 46) art. 1, comma 1, Aut. n. 080038 del 11/06/2008 - DCB Parma - Finito di stampare Dicembre 2008

Printed in December 2008

The contents of this Journal are indexed in PubMed - U.S. National Library of Medicine and Embase/Excerpta Medica

European Annals of Allergy and Clinical Immunology

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

Mattioli 1885
S.p.A - Strada Lodesana di Sopra - Loc. Vaio
43036 Fidenza (Parma) – Italy
Tel. 0039-(0)524-892111
Fax 0039-(0)524-892006
www.mattioli1885.com

General Management
President
Paolo Cioni

Editorial Management
Vice President and Journal Director
Federico Cioni

Editing Staff
Valeria Ceci
Natalie Cerioli
Cecilia Mutti
Anna Scotti

Marketing Management
Vice President and Marketing Director
Massimo Radaelli
Marketing Manager
Luca Ranzato
Marketing Secretary
Martine Brusini
Distribution Manager
Massimiliano Franzoni
Continuing Medical Education Manager
Simone Agnello

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 ad 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

Manuscripts should be sent to:
- **Prof. Alfred Sabbah** – 25, Av Jeanne d'Arc – F-49100 Angers – E-mail: alsabbah@wanadoo.fr
- **Dr. Gianenrico Senna** – Servizio di Allergologia, Ospedale Civile Maggiore – Piazza Stefani, 1 – I-37126 Verona – E-mail: gianenrico.senna@azosp.vr.it
- **Dr. Riccardo Asero** – Ambulatorio di Allergologia – Clinica S. Carlo – Via Ospedale, 21 – I-20037 Paderno Dugnano (MI) – E-mail: r.asero@libero.it
- **Dr. Carlo Lombardi** – Servizio di Allergologia, Unità Operativa di Medicina Generale, Ospedale Sant'Orsola-Fatebenefratelli – Via Vittorio Emanuele II, 27 – I-25122 Brescia – E-mail: hso.allergologia@fatebenefratelli.it

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 (SIUnits). 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.

All **plates** (except the first 4) are in principle to be paid for by the authors (whose property they remain), by prior agreement with the editors.
Two free issues will be sent to the author.

Mattioli 1885 S.p.A. Publishing House
Strada Lodesana di Sopra, Loc. Vaio
I - 43036 Fidenza – Italy
Tel. 0039-0524-892111
Fax 0039-(0)524-892006
E-mail: edit@mattioli1885.com

R. Asero[1], L. Jimeno[2], D. Barber[2]

Component-resolved diagnosis of plant food allergy by SPT

[1] Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano (MI), Italy;
[2] Departamento I + D, ALK-Abello, Madrid, Spain

Key words

Food allergy, fruits, vegetables, SPT, allergens

Summary

Background: *Fruits and vegetables may contain both labile and stable allergens. The former induce only OAS, whereas stable allergens may induce systemic reactions. Component-resolved diagnosis (CRD) of allergy to plant foods is therefore essential for the clinical management of allergic patients.* ***Methods:*** *80 adults allergic to plant foods underwent SPT with purified natural date palm profilin (Pho d 2), purified Mal d 1, a peach extract containing uniquely LTP, and with a kiwi extract containing uniquely stable allergens.* ***Results:*** *58 (72%) patients were monosensitized: 24 to Mal d 1, 24 to profilin, 7 to LTP, and 3 to kiwi. 22 patients were multi-sensitised: 14 to Mal d 1 and profilin, 2 to Mal d 1 and kiwi, 1 to LTP and profilin, 3 to LTP and Mal d 1, and 2 to LTP, Mal d 1 and profilin. Mal d 1 and LTP sensitisation were associated with apple and peach allergy, respectively, whereas profilin sensitisation was associated with allergy to melon, watermelon, banana, tomato and citrus fruits. 18/21 kiwi-allergic patients were sensitised to one of the cross-reacting allergens, but 2/18 reacted to kiwi-specific allergens as well.* ***Conclusions:*** *In patients with allergy to plant-derived foods CRD can be performed by SPT with purified allergen proteins. In the future, the availability of a larger number of purified natural or recombinant allergens for SPT will represent a simple means to classify food-allergic patients properly on the first visit.*

Introduction

Plant-derived foods represent by far the most frequent cause of food allergy in adults. One of the main features of fruits and vegetables as food allergens is that they may contain both labile and stable allergen proteins. This fact strongly influences the clinical presentation of allergy to a certain food as well as the risk associated with re-exposure to the offending food and/or to potentially cross-reactive ones. In effect, in virtually all sensitised patients pepsin-sensitive proteins induce only mild local symptoms (i.e., oral allergy syndrome, OAS), whereas more stable allergens reach the gastro-intestinal tract in a biologically active form and may induce potentially severe systemic symptoms (1-4). For the clinician this scenario is further complicated by the fact that, due to unclear reasons, subjects sensitised to stable allergens may have both mild (OAS) and/or systemic symptoms (5). The precise detection of the sensitizing allergen protein(s) in patients allergic to plant-derived foods is therefore extremely important to give patients correct advice about their clinical condition and about the necessity to exclude or not certain foods from their diet.

Fruits and vegetables contain 3 main, highly cross-reacting allergens two of which, namely profilin (6-9) and pro-

teins homologous to Bet v 1 (7,10,11), are extremely pepsin-sensitive whereas one, the non-specific lipid transfer protein (LTP), is very heat- and pepsin-stable (12-14). Up to now, in normal clinical settings the detection of sensitisation to these allergens has been based on indirect parameters such as the presence/absence of birch pollen hypersensitivity and/or the presence of hypersensitivity to many botanically unrelated pollens, suggestive of profilin hypersensitivity (these aspects have been reviewed in [15]). Another plant derived food that is causing an increasing number of allergies is kiwi. Again, prognosis of kiwi allergy is variable as allergic patients can be birch-pollen sensitised subjects (due to sensitisation to Bet v 1-homologous proteins) (16), profilin reactors (17), sensitised to kiwi-specific allergens (e.g. Act c 1) (18), or may have a latex-fruit syndrome (19); the 2 latter categories have theoretically a more risky clinical condition.

In recent years an increasing number of allergenic molecules have been sequenced and cloned, and are now available for routine in-vitro diagnostics; however, at the present most of these proteins are airborne allergens and most of the few available food allergens are of animal origin. The possibility to detect the sensitising allergen on the first visit in patients with a history of allergy to fruits and/or vegetable would represent a real step forward in the clinical practice both in terms of clinical care and of reduction of costs. The present study evaluated the effectiveness of component resolved diagnosis of plant-food allergy by means of SPT with extracts of plant-derived foods containing one single allergen protein due either to the loss of labile allergens during the preparation process, or to a proper purification procedure of the relevant protein.

Methods

Patients

80 consecutive patients (M/F 32/48; mean age 36 [SD 14.7] years, range 9-70 years) with a clinical history of allergy to plant derived foods confirmed by positive SPT with fresh offending food or with commercial food extract (1/20 wt/vol; ALK-Abello, Spain) seen at the allergy centre of the Clinica San Carlo were studied. A history of oral allergy syndrome (defined as itching of the oral mucosa and lips with or without angioedema immediately after eating specific foods), of urticaria with or without angioedema, and/or of severe gastrointestinal disorders following the ingestion of specific plant-derived foods was considered as compatible with food allergy and hence used as an inclusion criterion.

Skin tests

SPT with commercial extracts of pollens present in this geographical area, including grass, mugwort, ragweed, pellitory, plantain, birch, olive (all 50000 SBU/ml; Allergopharma, Reinbek, Germany) and cypress (30 HEP, ALK-Abello, Spain) were carried out in all patients. Further, all patients underwent SPT with purified natural date palm profilin (Pho d 2; 50 μg/ml; Alk Abello, Madrid, Spain; see beyond), with an apple extract containing uniquely Mal d 1 (2 μg/ml; ALK-Abello; see beyond), with a commercial peach extract containing uniquely lipid transfer protein (LTP 30 μg/ml; ALK-Abello)(15, 16), with a kiwi extract (5% w/v; ALK-Abello), and with a commercial natural rubber latex extract (500 μg protein/ml; ALK-Abello).

In a preliminary study on 36 patients with kiwi allergy positive on SPT with fresh kiwi by prick-prick technique the SPT with this commercial kiwi extract scored positive only in 8 cases, all without pollen-food allergy syndrome (5 patients were monosensitized to kiwi, 2 has latex-fruit allergy syndrome, and 1 patient was sensitized to LTP), whereas it did not induce any skin reaction in 28 patients with pollen-food allergy syndrome many of whom showing high levels of kiwi-specific IgE on CAP. It was therefore concluded that, similarly to apple extracts for SPT (10,20), this kiwi extract lacks both the allergen homologous to Bet v 1 and profilin, and contains uniquely stable allergens.

All SPT were performed using disposable 1 mm tip lancets (ALK-Abello). Readings were taken at 15 min, and a mean wheal diameter of 3 mm or more was considered positive (21). SPT with histamine 10 mg/ml and saline were carried out as positive and negative control, respectively.

Preparation of apple and Pho d 2 extracts

Lyophilized apple peels were extracted for 90 minutes at 4° with 0.1 mol/L sodium carbonate/bicarbonate, 0.1 mol/L Na Cl, pH 9.4. Extracts were centrifuged (1000 rpm, 30min, 4°C) and 50% glycerol was added. Apple extract contained 2 μg/ml of Mal d 1 as determined by ELISA, Mal d 4 < 0.1 μg/ml , Mal d 3 < 0.05 μg/ml. Natural profilin Pho d 2 was purified from date palm ex-

tract by affinity chromatography with a poly-L-proline-Sepharose (22); purity was checked by SDS PAGE, mass spectrometry and amino acid analysis. The concentration of Pho d 2 in the extract was 50 µg/ml

Statistics

Associations were assessed by the chi-square test with Yates' correction. P values < 0.05 were considered statistically significant.

Results

Clinical presentation of food allergy

All patients reported oral allergy syndrome as the only symptom of food allergy except 4 who also had urticaria (2 cases), asthma, rhinitis and gastro enteric symptoms (1 case) and gastric pain (1 case).

SPT with food allergens and clinical associations

All 80 patients scored positive with at least one out of 4 allergens tested (namely, profilin, Mal d 1, LTP, and kiwi-specific allergens). Results are summarized in table 1. Fifty-eight/80 (72%) patients turned out to be monosensitised (24 to profilin, 24 to Mal d 1, 7 to LTP, and 3 to kiwi), whereas 22 were sensitised to > 1 allergen (14 Mal d 1 + profilin; 3 Mal d 1 + LTP; 2 Mal d 1 + Kiwi; 2 Mal d 1 + profilin + LTP; and 1 profilin + LTP). No patient scored positive on SPT with latex extract.

In patients sensitised to the 3 cross-reacting allergens (Mal d 1, profilin, and LTP) the pattern of offending foods changed with the sensitising allergen (Tab. 2). Apple allergy was significantly associated with sensitisation to Mal d 1; 30/45 (67%) Mal d 1-hypersensitive subjects had apple allergy ($p < 0.05$). Peach allergy was associated with sensitisation to lipid transfer protein; 12/13 (92%) LTP-hypersensitive patients were allergic to this fruit ($p < 0.05$). Finally allergy to melon, watermelon, citrus fruit, banana, and tomato was significantly associated with profilin sensitisation; of 41 profilin-hypersensitive patients 24 (58%), 14(34%), 19 (46%), 10 (24%), and 9 (22%) were allergic to melon, watermelon, tomato, banana, and citrus fruits, respectively ($p < 0.05$-0.001).

The 3 patients reporting oral allergy syndrome plus food-induced urticaria (n = 2) or plus asthma, rhinitis, and gastro enteric symptoms (n = 1) were sensitised to LTP. The patient reporting OAS and gastric pain was monosensitised to kiwi. Clinically, patients sensitised to kiwi-specific proteins reported a much more severe oral symptoms (frequently associated with oedema of the lips, tongue, and pharynx and with tightness of throat) than kiwi-allergic patients sensitised to Mal d 1 and/or profilin.

Association with pollen hypersensitivity

Hypersensitivity to seasonal airborne allergens tested is shown in table 3. Not surprisingly, all Mal d 1-sensitized patients were allergic to birch pollen, and profilin-hypersensitive patients were sensitised to most pollens, with the partial exception of Parietaria and cypress. Most LTP-allergic patients were sensitised to grass pollen. In contrast, patients monosensitized to kiwi were not sensitised to seasonal allergens with the exception of one subject sensitised to grass pollen.

Table 1 - Skin reactivity and clinical symptoms in 80 consecutive patients with plant-food allergy

Skin reactivity	No.	OAS	OAS + urticaria	OAS + RAG	OAS + G
Mal d 1 only	24	24			
Profilin only	24	24			
LTP only	7	6		1	
Mal d 1 + Profilin	14	14			
Profilin + LTP	1		1		
Mal d 1 + Profilin + LTP	2	2			
Mal d 1 + LTP	3	2	1		
Mal d 1 + Kiwi	2	2			
Kiwi only	3	2			1

R: rhinitis; A: asthma; G: gastrointestinal

Table 2 - Offending foods and statistical associations in 80 patients allergic to plant-derived foods sensitised to different allergen proteins

	M	P	L	MP	PL	MPL	ML	MK	K	p		
No. Patients	24	24	7	14	1	2	3	2	3	M	P	L
Apple	16	9	2	10	1	1	3			<0.05	NS	NS
Pear	8	4	0	3		1	1			NS	NS	NS
Peach	12	12	7	7	1	1	2			NS	NS	<0.05
Cherry	12	7	1	2		1	3			NS	NS	NS
Plum	6	3	0	3		1	0			NS	NS	NS
Apricot	7	6	0	6		0	2			NS	NS	NS
Medlar	2	0	0	1		0	0			NS	NS	NS
Almond	4	2	1	0		0	0			NS	NS	NS
Hazelnut	10	8	3	6		1	1			NS	NS	NS
Walnut	6	6	2	1		1	0			NS	NS	NS
Peanut		2	2	0		0	0			NS	NS	NS
Melon	2	17	0	6		1	0			NS	<0.001	NS
Watermelon	1	10	0	3		1	0			NS	<0.01	NS
Orange/tang	1	7	0	1		1	0			NS	<0.05	NS
Grapes	0	2	0	1		1	0			NS	NS	NS
Pineapple	1	4	1	2		0	0			NS	NS	NS
Banana	0	8	0	2		0	0			NS	<0.01	NS
Carrot	7	3	0	3		0	0			NS	NS	NS
Celery	4	2	0	1		0	0			NS	NS	NS
Fennel	4	2	1	2		0	1			NS	NS	NS
Tomato	1	12	1	5		2	0			NS	<0.001	NS
Eggplant	1	2	1	0		0	0			NS	NS	NS
Lettuce	0	1	0	0		0	0			NS	NS	NS
Kiwi	4	4	2	5		0	1	2	3	NS	NS	NS
Strawberry	2	1	1	3		0	0			NS	NS	NS
Zucchini	0	1	0	0		0	0			NS	NS	NS
Persimmon	0	1	0	0		1	0			NS	NS	NS
Coconut	0	1	1	0		0	0			NS	NS	NS

M: Mal d 1; P: Profilin; L: Lipid transfer protein; K: Kiwi specific allergens

Discussion

The present study highlights the importance of being able to carry out a component-resolved diagnosis in-vivo when the patient with plant food allergy is seen for the first time in the clinic. In keeping with previous studies, all patients sensitised to labile allergens had oral allergy syndrome as the only clinical expression of their food allergy (10,23); however, also most of the patients sensitised to stable vegetable food allergens in this study reported oral allergy syndrome. Further, 22/80 (28%) patients were multi-sensitised to vegetable food allergens, and 8 of these subjects reacted to both labile (Mal d 1 and/or profilin) and stable (LTP and/or kiwi) allergen proteins. In these patients component-resolved diagnosis has been essential to give the correct advice about the possibility to maintain (e.g., in subjects sensitised to labile proteins) or the necessity to change dietary habits (e.g. avoidance of kiwi fruit in specifically sensitised subjects; avoidance of whole fresh Rosaceae with or without tree nuts, as well as avoidance of commercial Rosaceae fruit juices in LTP-allergic patients), or how to reduce allergenicity of potentially offending foods (e.g. to try peeled Rosaceae fruits in LTP-allergic subjects [24]; to eat fruit salads and drink

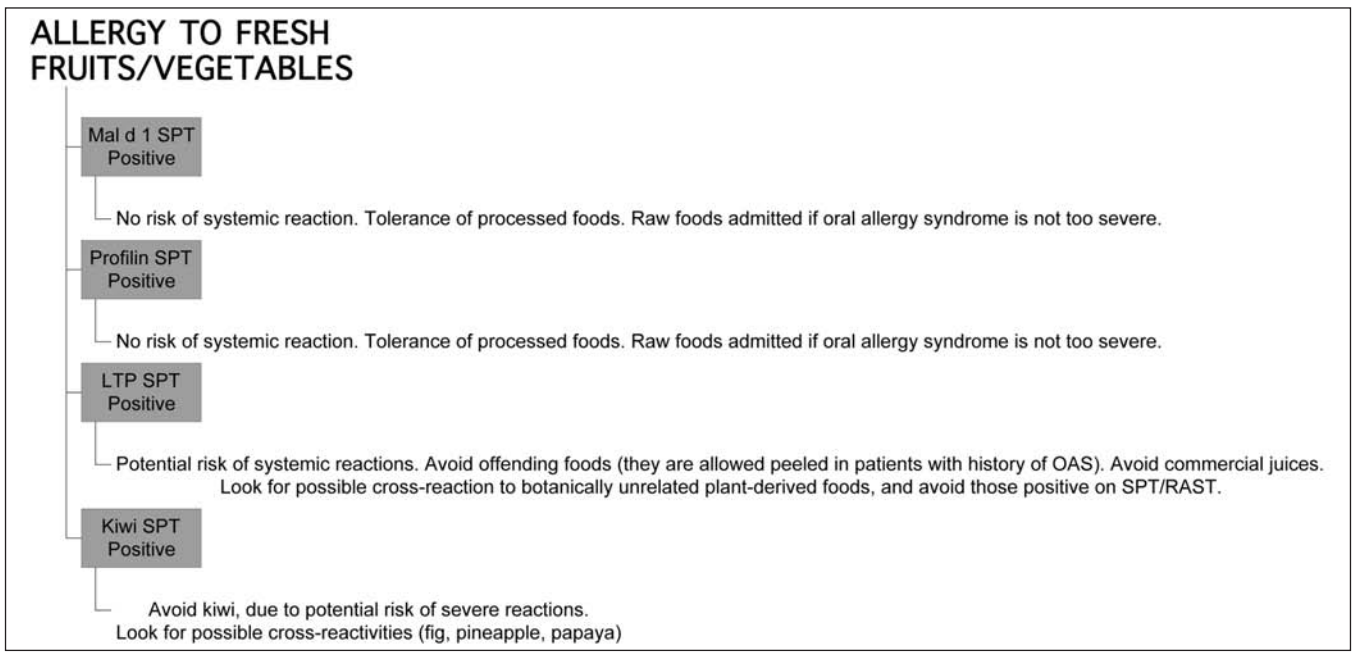
Table 3 - Sensitivity to pollens in 80 patients allergic to plant foods

Sensitivity	Grass	Mugwort	Ragweed	Pellitory	Plantain	Birch	Olive	Cypress
Mal d 1 only (n=24)	11 (46%)	8 (33%)	12 (50%)	4 (17%)	5 (21%)	24 (100%)	9 (38%)	10 (42%)
Profilin only (n= 24)	24 (100%)	22 (92%)	23 (96%)	10 (42%)	21 (88%)	23 (96%)	23 (96%)	10 (42%)
LTP only (n= 7)	6 (86%)	1 (14%)	3 (43%)	0 (0%)	0 (0%)	1 (14%)	0 (0%)	0 (0%)
Mal d 1+Profilin (n=14)	14 (100%)	13 (93%)	13 (93%)	9 (64%)	14 (100%)	14 (100%)	13 (93%)	8 (57%)
Profilin + LTP (n=1)	1 (100%)	1 (100%)	1 (100%)	0 (0%)	1 (100 %)	1 (100%)	1 (100%)	0 (0%)
Mal d 1 + profilin + LTP (n=2)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	0 (0%)
Mal d 1 + LTP (n= 3)	3 (100%)	0 (0%)	1 (33%)	1 (33%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)
Mal d 1 + Kiwi (n= 2)	2 (100%)	1 (50%)	2 (100%)	1 (50%)	2 (100%)	2 (100%)	2 (100%)	0 (0%)
Kiwi (n=3)	1 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total Mal d 1 (n= 45)	32 (71%)	24 (53%)	30 (67%)	17 (38%)	23 (51%)	45 (100%)	26 (58%)	18 (40%)
Total Profilin (n= 41)	41 (100%)	38 (93%)	39 (95%)	21 (51%)	38 (93%)	40 (98%)	39 (95%)	18 (44%)
Total LTP (n=13)	12 (92%)	4 (31%)	7 (54%)	3 (23%)	3 (23%)	7 (54%)	3 (23%)	0 (0%)

commercial fruit juices in patients sensitised to labile allergens [15]). The validity of the diagnostic means used in this study is further, indirectly confirmed by the observed associations. Apple has been known as the clinical marker of birch/food allergy syndrome for a long time (25); similarly, peach allergy is a "trademark" of LTP hypersensitivity (26, 27), and the association between profilin sensitisation and allergy to melon, watermelon, citrus fruit, banana, and tomato is in keeping with previous studies (28, 29).

The panel of plant-food allergens used in this study was obviously incomplete as relevant cross-reacting allergens

Figure 1 - Allergy to fresh fruits/vegetables



such as the seed storage proteins, including 2S-albumins, vicilins, and legumins, were missing. Nonetheless, no patients out of the 80 consecutive ones included in the present study reported a history of systemic reactions to tree nuts and/or seeds, which suggests that (at least in this area) allergy to seed storage proteins is much less frequent than allergy to Bet v 1-homologue proteins, profilins, LTP or kiwi. Hopefully, these purified proteins as well will be available for in-vivo testing in the future. A summary of the clinical use of these 4 allergens is suggested in figure 1.

One further aspect that is worth discussing is the advantage of performing SPT with purified proteins rather that carrying out a molecular analysis by in-vitro tests. Presently, both the immunoCAP (Phadia, Uppsala, Sweden) and the protein micro-array ISAC (VBC Genomics- Phadia) include recombinant profilin, Bet v 1-homologous proteins and LTP (Pru p 3 and others) in their panels. The latter also includes kiwi-specific allergens. However, both assays are more expensive than a simple SPT. Particularly, the micro-array is a "take it or leave it" test in which one is forced to measure IgE specific for > 90 allergen proteins, even if the diagnostic question deals with 3-4 allergens; the immunoCAP is still unable to produce a differential diagnosis between primary or secondary kiwi allergy. Finally, the results that both assay produce are not readily available. The immunoblot analysis is another common means to investigate allergenic proteins in-vitro, but it is still not available in most clinical settings.

In conclusion, in recent years molecular biology techniques have much improved the diagnosis of allergy, and several allergen proteins are already available for in-vitro assays, although the number of food allergens still remains limited. Purified food allergen proteins are being (slowly) introduced also for in-vivo testing and this will enormously simplify doctors' work and improve patients' care.

References

1. Scheurer, Lauer I, Foetisch K, et al. Strong allergenicity of Pru av 3, the lipid transfer protein from cherry, is related to high stability against thermal processing and digestion. J Allergy Clin Immunol 2004; 114: 900-7.
2. Akkerdaas JH, Wensing M, Asero R, et al. IgE binding to pepsin-digested food extracts. Int Arch Allergy Immunol 2005; 138: 203-8.
3. Lehmann K, Schweimer K, Reese G, et al. Structure and stability of 2S albumin-type peanut allergens: implications to the severity of peanut allergic reactions. Biochem J 2006; 395: 463-72.
4. Bowman CC, Selgrade MK. Differences in allergenic potential of food extracts following oral exposure in mice reflect differences in digestibility: potential approaches to safety assessment. Toxicol Sci 2007; Epub ahead of print.
5. Asero R. Detection and clinical characterization of patients with oral allergy syndrome caused by stable allergens in Rosaceae and nuts. Ann Allergy Asthma Immunol 1999; 83: 377-83 .
6. Valenta R, Duchene M, Ebner C, Valent P, et al. Profilins constitute a novel family of functional plant pan-allergens. J Exp Med 1992; 175: 377-85.
7. Ebner C, Hirschwehr R, Bauer L, et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). J Allergy Clin Immunol 1995; 95: 962-9.
8. Van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reacting allergen in pollen and vegetable food. Int Arch Allergy Imunol 1992; 98: 97-104.
9. Rodriguez-Perez R, Crespo JF, Rodriguez J, Salcedo G. Profilin is a relevant melon allergen susceptible to pepsin digestion in patients with oral allergy syndrome. J Allergy Clin Immunol 2003; 111: 634-9.
10. Bjorksten F, Halmepuro L, Hannuksela M. Extraction and properties of apple allergens. Allergy 1980; 35: 671-7.
11. Rudeschko O, Fahlbusch B, Henzgen M, et al. Investigation of the stability of apple allergen extracts. Allergy 1995; 50: 575-9.
12. Asero R, Mistrello G, Roncarolo D, et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. Int Arch Allergy Immunol 2000; 122: 20-32.
13. Asero R, Mistrello G, Roncarolo D, Amato S, Falagiani P. Analysis of the heat stability of lipid transfer protein from apple. J Allergy Clin Immunol 2003; 112: 1009-11.
14. Sancho AI, Rigby NM, Zuidmeer L, et al The effect of thermal processing on the IgE reactivity of the non-specific lipid transfer protein from apple, Mal d 3. Allergy 2005; 60 (10): 1262-8.
15. Asero R. Plant food allergies: a suggested approach to allergen-resolved diagnosis in the clinical practice by identifying easily available sensitization markers. Int Arch Allergy Immunol 2005; 138: 1-11.
16. Gall H, Kalveram KJ, Forck G, Sterry W. Kiwi fruit allergy: a new birch pollen-associated food allergy. J Allergy Clin Immunol 1994; 94: 70-6.
17. Rudeschko O, Fahlbusch B Steurich F, Schlenvoigt G, Jager L. Kiwi allergens and their cross-reactivity with birch, rye, timothy, and mugwort pollen. J Investig Allergol Clin Immunol 1998; 8: 78-84.
18. Alemán A, Sastre J, Quirce S, et al. Allergy to kiwi: a double-blind, placebo-controlled food challenge study in patients from a birch-free area. J Allergy Clin Immunol 2004; 113: 543-50.
19. Blanco C. Latex-fruit allergy syndrome. Curr Allergy Asthma Rep 2003; 3: 47-53.
20. Ortolani C, Ispano M, Pastorello EA, Ansaloni R, Magri GC. Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. J Allergy Clin Immunol 1989; 83 (3):683-90.
21. Dreborg S, Frew A. Allergen standardization and skin tests. Allergy 1993; 48: 49-75.
22. Asturias JA, Ibarrola I, Fernandez J, Arilla MC, Gonzalez-Rioja R, Martinez A. Pho d 2, a major allergen from date palm pollen, is

a profilin: cloning, sequencing, and immunoglobulin E cross-reactivity with other profilins. Clin Exp Allergy 2005; 35: 374-81.
23. Amlot P, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS) symptoms of IgE-mediated hypersensitivity to foods. Clin Allergy 1987; 17: 33-42.
24. Fernandez-Rivas M, Cuevas M. Peels of Rosaceae have a higher allergenicity than pulps. Clin Exp Allergy 1999; 29: 1239-47.
25. Lahti A, Bjorksten F, Hannuksela M. Allergy to birch pollen and apple, and cross-reactivity of the allergens studied with the RAST. Allergy 1980; 35: 297-300.
26. Lleonart R, Cisterò A, Carreira J, Batista A, Moscoso del Prado J. Food allergy: identification of the major IgE-binding of peach (Prunus persica). Ann Allergy 1992; 69: 128-30.
27. Asero R, Mistrello G, Roncarolo D, Amato S. Relationship between peach lipid transfer protein IgE levels and hypersensitivity to non-Rosaceae vegetable foods in patients allergic to lipid transfer protein. Ann Allergy Asthma Immunol 2004; 92: 268-72.
28. Asero R, Mistrello G, Roncarolo D, Amato S, Zanoni D, Barocci F, Caldironi G. Detection of clinical markers of sensitisation to profilin in patients allergic to plant-derived foods. J Allergy Clin Immunol 2003; 112: 427-32.
29. Asero R, Monsalve G, Barber D. Profilin sensitisation detected in the office by SPT: a study of prevalence and clinical relevance of profilin as a plant food allergen. Clin Exp Allergy 2008; 38: 1033-7.

C. D'Antonio[1], M. Galimberti[1], B. Barbone[1], M. Calamari[1], G. Airoldi[2], M. Campanini[2], C. Di Pietrantonj[3], G.C. Avanzi[1]

Suspected acute allergic reactions: analysis of admissions to the Emergency Department of the AOU Maggiore della Carità Hospital in Novara from 2003 to 2007

[1] Eastern Piedmont University, Department of Clinical and Experimental Medicine, Allergology and Immunology Unit, Director Prof . Avanzi Gian Carlo MD
[2] Internal and Emergency Medicine, Director Campanini Mauro MD –"Maggiore Hospital" - Novara.
[3] Epidemiological Regional Center for surveillance, prevention and monitoring of infectious diseases (SEREMI) – ASR 20 Alessandria

Key words

Emergency Department, acute allergic reaction, anaphylaxis, medical admission, triage code

Summary

Objective of the Study: *The aim of our work is to ascertain the frequency and the impact of acute allergic reactions on the routine of a highly-specialized Emergency Department collecting information on the admission, the typology of symptoms and the degree of severity calculating the incidence and the outcomes of the events.* ***Materials and methods:*** *The study started the 1 July 2006 and the records of the Emergency Department of the Maggiore della Carità Hospital in Novara were consulted retrospectively in the period between the 1 January 2003 and the 31 December 2006, and prospectively up to the 31 December 2007, using keywords that could identify admission for suspected allergic reactions. Information relating to internal medicine and/or pediatric cases were examined, excluding all surgical and/or trauma cases. The number of admissions per year was considered broken down by clinical signs, triage assessment upon admission and discharge outcome.* ***Results:*** *Admissions to the Emergency Department during the period under consideration were 165,120 with 6,107 suspected cases of allergic reactions. The symptoms most frequently reported both in adults (A) and children (C ≤18 years old), were: hives 37%, asthma 20.65 (A)% and 27,4% (C); drug allergy 7.5% (A) and 6,1% (C). Reactions to Hymenoptera venom were less frequent, 4.7% (A) and 1.27% (C); the frequency of angioedema, conjunctivitis and rhinitis was between 1 and 4%. The incidence of food allergies (1.4%) and anaphylaxis (0.8%) was comparable for all ages. The triage assessment showed a significant percentage of "yellow" and "red" codes, with 362 cases (5.9%) and 71 cases (1.16%) respectively. A total of 151 patients was hospitalized, no one classified as "white" code. Death occurred in 7 cases: 4 "yellow" codes and 3 "red" codes, respectively. A more detailed specialistic evaluation was recommended in only 10% of the patients.* ***Conclusions:*** *Admissions to the Emergency Department for suspected allergic reaction are proportional to the number of overall admissions for internal medicine cases and do not appear to be related to the general increase of allergies in the population. This led us to focus our attention on how allergic diseases impact the work of an Emergency*

Department and how to describe the discharge diagnosis better. A significant number of descriptive diagnoses also turned out to be inaccurate and did not allow the syndrome to be identified properly. The analysis of this information aims to be a stimulus to improve the emergency clinical approach used for allergic diseases and to plan the adequate management of allergic patients after they have been treated in hospital.

Foreword

The increased frequency of allergic diseases in the general population is shown in epidemiological studies conducted by the international scientific community.
In industrialized countries, about 20-25% of the population has respiratory tract symptoms and the incidence of these allergic syndromes in European countries (United Kingdom, Germany, Switzerland, Finland) ranges between 22 and 35% (1-3). The incidence in western countries appears higher in the 18-34 age group and in the 35-49 age group, with a reduction in the number of cases after 50 years of age (4).
The number of cases of asthma has changed over the years: from 10% estimated in the USA in the 90s to more than 15% in California alone (5,6).
Italian incidence of respiratory allergies shows a high heterogeneity: in a study conducted in Liguria, the estimated incidence of allergic rhinitis ranges between 1.54% in 1983 and 2.2% in 1995 (7); for asthma, the estimated incidence in Italy was about 3.3-5.5% in the 80s, while more recent figures show an incidence between 4% to 7% (8,9).
An Italian study on asthma (10) shows a 9.5% incidence of asthma in children aged 6 years old, and 10.4% in adolescents aged 13-14 years old. The incidence of rhinitis in children aged 6 is 9%, and in adolescents aged 13-14 is 17.2%, and for eczema it is 17% in children aged 6 and 12.8% in adolescents.
Although people who claim to suffer from food-related adverse events is approximately 20% of the population in Europe (11), the true incidence of food allergies is 2-4% of the adult population and approximately 5% of children (12,13). The frequency of allergic reactions to food additives does not exceed 0.5% of cases (12,13).
Epidemiological findings on reactions to drugs are still uncertain. However, only more rarely the reactions are unexpected and potentially severe (14,15). Unforeseeable reactions, whether allergic or pseudo allergic, account for no more than 5-10% of the total amount.
Allergic symptoms are mainly mild/moderate and are usually treated in specialist practices, rarely the severity of the symptoms requires intensive care (16,1,). Two syndromes are particularly dangerous: persistent severe asthma and anaphylaxis, since they can cause the patient's death in a few minutes (18-22).
Furthermore, after the acute phase has been treated, patients with the most severe symptoms, who have perhaps been treated in the Intensive Care Unit, are not always sufficiently made aware of the need to refer to specialist allergy centers for follow-up treatment (23,24).
On the other hand the American Academy of Allergy Asthma & Immunology recommends referring all patients to an allergist, especially children, for an in-depth diagnostic-therapeutic classification (25).
The aim of our work is to ascertain the frequency and resulting impact of acute allergic reactions on the routine of a highly-specialized Emergency Department collecting information on admission, typology of symptoms, the degree of severity, calculating the incidence and the outcomes of the events.
The analysis of this information aims to be a stimulus to improve the emergency clinical approach used for allergic diseases and to plan the adequate management of allergic patients after they have been treated in hospital.

Materials and methods

The study started at 1 July 2006 examines retrospectively the discharge records of the Emergency Department (E.D.) at the Azienda Ospedaliera - Universitaria "Maggiore della Carità" Hospital in Novara, (city of Piedmont Region in northern Italy), up to 31 December 2006 and prospectively up to 31 December 2007, using either single "keywords" or in various associations in the Emergency Department discharge diagnosis: allergy, food allergy, anaphylaxis, angioedema, asthma, conjunctivitis, dermatitis, dyspnea, edema, insect sting–induced dermatitis, rash, Hymenoptera, hypotension, rhinoconjunctivitis, hives, reaction, shock.
The records were examined for each single year and for the entire period, considering two age groups, adults (>18 years (A)) and children (≤ 18 years(C)).

The target population was the patients admitted to the Emergency Department where their diagnoses were coded using the ICD9CM.
Admission to the various rooms is established under the triage assessment made by the nursing staff (Tab. 1).
The diagnoses and triage code assigned to the patients were assessed and compared to diagnoses and triage code assigned at the discharge. The patients were subsequently classified into: ordinary discharges, voluntary discharges (leaving against the physician's advice), discharges with urgent follow-up treatment in clinics, discharges after having been kept temporarily under observation, hospitalization after a short period under observation at the E.D. and death.
Confidence interval for prevalence rate was computed by Wilson and Newcombe methods (36), a graphical display was performed using simple correspondence analysis to study and visualize the link between triage code and discharge patients categories (37).

Table 1 – Nurse triage codes

Red code is assigned to patients with critical vital functions that are immediately life-threatening or that could severely damage a vital organ.	**Yellow** code is assigned to patients whose vital functions are unstable, whose conditions could deteriorate quickly and are potentially life-threatening or could severely damage a vital organ within minutes or in the following hours. This includes moderate to severe asthma and generalized, ingravescent hives-angioedema.	**Green** code is assigned to patients whose vital functions are stable and where the risk to their lives or damage to their vital organs within 6-12 hours is minimal. This includes mild-moderate bronchial asthma and skin reactions such as atopic eczema and acute hives without any signs of evolution.	**White** code is assigned to patients whose vital functions are stable, who suffer from chronic or mild diseases, where the risk to their lives or damage to their vital organs within 24 hours is minimal. This includes allergic diseases such as mild to moderate sudden-onset oculorhinitis and sub-acute or recurring hives.
dyspnea: (at rest) with spO_2 < 90% (in non-COPD patient), RR > 30 /min or cyanosis **cardio-circulatory abnormalities**: sweating or paleness (with HR < 50 bpm and > 170 bpm, systolic BP < 80 mmHg and > 200 mmHg) **skin and mucosal abnormalities**: cutaneous marbling and cyanosis **other problems**: breathing with RR < 12 acts/min, diagnostic ECG for AMI or VT **negative for**: hemorrhage, pain, traumas, neurological and psychic disorders	**dyspnea**: (at rest) with gasping or wheezing, difficult (wheezy) breathing, sweating or paleness, spO_2 < 90 (COPD), asthmatic, ischemic heart disease, BP > 180/120 mmHg, suspected allergic reaction **cardio-circulatory abnormalities**: sweating or paleness (with HR > 50 and < 60 bpm, systolic BP > 80 and < 90 mmHg and > 180 and < 200 mmHg), no sweating or paleness upon arrival at the E.D (with HR < 50 bpm and > 170 bpm, systolic BP < 80 mmHg and > 200 mmHg) **skin and mucosal abnormalities**: lip/tongue edema from suspected allergy, **negative for**: hemorrhage, pain, traumas, neurological and psychic disorders, other problems	**cardio-circulatory abnormalities**: sweating or paleness (with HR > 60 and < 120 bpm, systolic BP > 90 and < 180 mmHg), no sweating or paleness upon arrival at the E.D. (with HR > 50 and < 60 bpm and > 120 and < 170 bpm, systolic BP > 80 and < 90 mmHg and > 180 < 200 mmHg) **skin and mucosal abnormalities**: bilateral edema of the limbs (main reason for admission to the ED), periorbital or facial edema **negative for**: dyspnea (at rest), hemorrhage, pain, traumas, neurological and psychic disorders, other problems	**Rhinitis**: from mild to moderate **skin and mucosal abnormalities**: hives in the sub-acute phase **negative for**: dyspnea, cardio-circulatory abnormalities, neurological and psychic disorders, hemorrhage, pain, traumas, other problems

Results

The medical admissions during the period considered were 165,120, the suspected allergic reactions were 6,107 (3.7%), for a potential population of approximately around 250,000 inhabitants (5.87% of 4,256,451 inhabitants in Piedmont).
The admissions by a single patient for the same condition was counted only once. On average, 8 persons per year were treated for the same symptoms by the Emergency Department two or more times.
Table 2 is a list of admissions to the Emergency Department per year for suspected allergies in the various age groups, totally 71,25% are adults and 28,75% are children.
The table 2 shows a high variation of the numbers of cases of allergic diseases in pediatric patients and a subsequently variation of the percentage. Moreover, the data show a lower number of cases in 2005 than in the other years in pediatric; a lower number of admission in 2007 both in adults and in pediatric patients, and we can also observe a lower number of cases in pediatric.
However in the four years period the impact of suspected allergy is around 4% of the total admissions at ED.
The most frequently recurring allergic syndromes for each age group are shown in table 3: Hives, Asthma, Insect sting-induced dermatitis, General Allergy and Drug Allergy represent the 77,7% of the total allergic disease in adults and 87.5% in children.
In particular among children the insect sting-induced dermatitis is 14% of the total diagnoses, against 6.81% in the adults: this difference is statistically significant. This high prevalence lies mainly in the diagnosis performed in 2005.
In table 3 we can observe two different clusters of allergies that show a difference rate (between adult and children) statistically significant: the first cluster includes Hives, Asthma and Insect sting-induced dermatitis and shows a high prevalence in children, the second cluster includes Hymenoptera allergy, Angioedema and Solar Dermatitis and shows a high prevalence in adults. Less frequent causes for urgent treatment were reactions to hymenoptera venom, angioedema, conjunctivitis and rhinitis.
To understand how suspect allergy is managed in Emergency Department we have compared discharge types (Normal, Urgent specialist follow-up, After short observation, Voluntary, Hospitalization and Death) to triage code assigned at patient admission.
During the four years of observation we considered 6107 cases: Normal discharge in 5053 cases (82.8%), urgent specialist follow up in 12.1%, Voluntary discharge after short observation ranged from 1.1% to 1.4%, Hospitalization after ED 2.4% and Death at the ED 0.15%. White code occurred in 60.3%, Green code 32.6%, Yellow code 5.9% and Red code 1.2%.
The simultaneous analysis of the triage codes and patients discharge categories displayed in figure 1 shows three clusters of discharge type "Normal Discharge", "Discharge after observation or voluntary or specialist" and "Death or Hospitalization". This analysis allows the characterization of the three clusters: the Normal discharge is characterized by white triage code, the second cluster is mainly characterized by Green and Yellow codes and the third is characterized by Red and Yellow codes.
The Normal discharged patients present the less severe

Table 2 - Admission to the ED for year

Year	Adult			95% CI		Pediatric			95% CI		Total			95% CI	
	a	n	%	LL	UL	a	n	%	LL	UL	a	n	%	LL	UL
2003	832	22657	3.67%	3.43%	3.92%	481	7173	6.71%	6.13%	7.28%	1313	29830	4.40%	4.17%	4.63%
2004	767	25398	3.02%	2.81%	3.23%	580	10212	5.68%	5.23%	6.13%	1347	35610	3.78%	3.58%	3.98%
2005	724	27505	2.63%	2.44%	2.82%	274	11541	2.37%	2.10%	2.65%	998	39046	2.56%	2.40%	2.71%
2006	869	28310	3.07%	2.87%	3.27%	629	12994	4.84%	4.47%	5.21%	1498	41304	3.63%	3.45%	3.81%
2007	904	18961	4.77%	4.46%	5.07%	47	369	12.74%	9.34%	16.14%	951	19330	4.92%	4.61%	5.22%
Total	4096	122831	3.33%	3.23%	3.44%	2011	42289	4.76%	4.55%	4.96%	6107	165120	3.70%	3.61%	3.79%

(a): suspected allergic diseases
(n): number of emergency admissions
(%) percentage is compute on total admission per emergency (Medical or Pediatric respectively) and year of study
CI: Confidence Interval; LL: Lower confidence Limit, UL: Upper confidence Limit

Table 3 - Allergic syndromes 2003-2007

	Adults	%	rate x 1000	LL	UL	Children	%	rate x 1000	LL	UL	Delta	LL	UL	sign
Hives	1384	33.79%	11.27	10.69	11.87	712	35.41%	16.84	15.65	18.11	-5.57	-6.96	-4.24	*
Asthma	883	21.56%	7.19	6.73	7.68	547	27.20%	12.93	11.90	14.06	-5.75	-6.96	-4.60	*
Entomodermatosis	279	6.81%	2.27	2.02	2.55	288	14.32%	6.81	6.07	7.64	-4.54	-5.41	-3.75	*
General allergy	330	8.06%	2.69	2.41	2.99	92	4.57%	2.18	1.77	2.67	0.51	-0.05	1.02	
Drug allergy	305	7.45%	2.48	2.22	2.78	120	5.97%	2.84	2.37	3.39	-0.35	-0.97	0.19	
Conjunctivitis	160	3.91%	1.30	1.12	1.52	64	3.18%	1.51	1.19	1.93	-0.21	-0.67	0.18	
Hymenopter allergy	171	4.17%	1.39	1.20	1.62	25	1.24%	0.59	0.40	0.87	0.80	0.46	1.10	*
Angioedema	196	4.79%	1.60	1.39	1.84	45	2.24%	1.06	0.80	1.42	0.53	0.12	0.89	*
Dermatitis (CD +eczema)	193	4.71%	1.57	1.36	1.81	60	2.98%	1.42	1.10	1.83	0.15	-0.30	0.55	
Food allergy	62	1.51%	0.50	0.39	0.65	24	1.19%	0.57	0.38	0.84	-0.06	-0.36	0.17	
Rhinoconjunctivitis	70	1.71%	0.57	0.45	0.72	18	0.90%	0.43	0.27	0.67	0.14	-0.13	0.36	
Shock	31	0.76%	0.25	0.18	0.36	16	0.80%	0.38	0.23	0.61	-0.13	-0.37	0.05	
Solar dermatitis	32	0.78%	0.26	0.18	0.37	0	0.00%	0.00	0.00	0.09	0.26	0.14	0.37	*
Total	4096					2011								
Total: Admission 2003-2007	122831					42289								

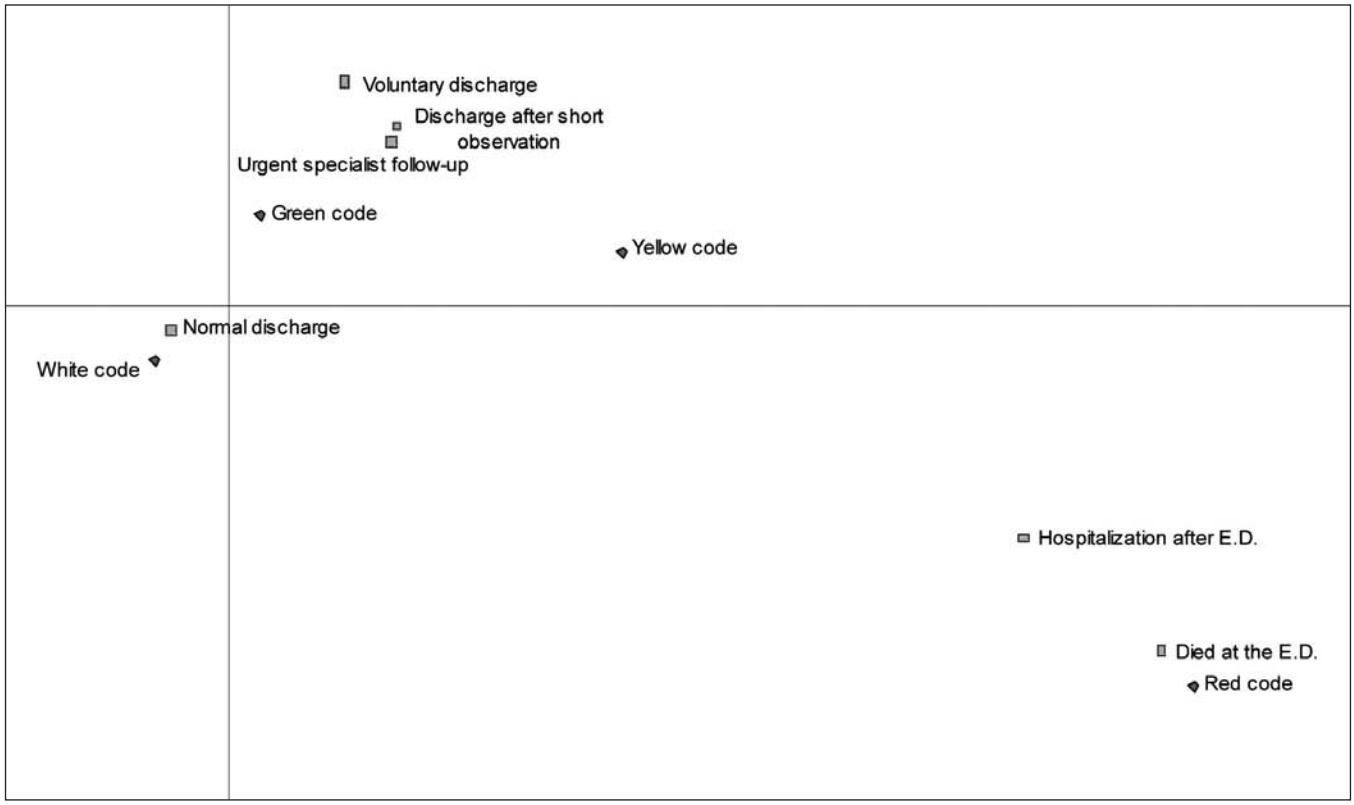
(%): percentage is computed using as denominator total allergies; (rate x 1000): is computed using as denominator total admission 2003-2007; (CD) : contact dermatitis; (rd): rate difference between Adult and Children; (95% CI): 95% Confidence Interval; (LL): Low confidence Limit: (UL): Upper confidence Limit: (Sign.): rd statistical significant at 5% level

codes: 3602 "white" codes, 1361 "green" codes, 90 "yellow" codes and no "red" codes.
The Voluntary discharges against the physician's opinion present mainly mild severe codes (2 white codes, 58 Green codes and 11 Yellow codes and no Red codes).
The Discharges with referral to a specialist practice present mainly mild severe codes: 79 White codes, 482 green codes, 163 Yellow codes and 13 Red codes.
The Discharges after a short period of observation present no White codes, 73 Green codes, 11 Yellow codes and 4 Red codes.
The Hospitalization after ED presents 0 White codes, 17 Green Codes, 83 Yellow codes 51 Red codes. Death at the ED were 0 White code, 0 Green codes, 4 Yellow codes and 3 Red codes. Four adults died at the E.D for anaphylaxis, 2 "yellow" codes and 2 "red" codes.
Exacerbated bronchial asthma led to the death of 2 people, 1 adult and 1 child, while the death of one child, who was a "yellow" code at the admission as insect sting-induced dermatitis, was probably a case of anaphylaxis from a hymenopter sting.
These data endorse a little evidence on the appropriate management of the suspected allergic diseases at the ED.

Discussion

The impact of the cases of suspected allergy represents an amount not so indifferent on the total amount of the admissions at the E. D.
The number of admissions to the Emergency Department for suspected allergic diseases varied between 2.6% and 4.9% (media 3.7%) of the total during the years considered.
Therefore no higher incidence of acute onset of "allergic reactions" was reported, despite the increase in allergic diseases among the general population (4,7,10).
This could be the result of the role of prevention, and the diagnostic and therapeutic work performed by the Regional Hospital Network for Allergic Diseases, a computerized and well-established network in the territory (24).
The data of the E.D. are important really for their role of access to the sanitary services. In addition, the nature - not so serious - of the cases let them get away from the epidemiological monitoring, even in the presence of an informative web/network of allergology.
In fact less then 20% of the patients examined and treated at the E.D. (542 cases) were given a written referral for

Figure 1 – Simple Correspondence Analysis performs a simultaneous plot of the triage codes and patients discharge categories. The quality of representation is 99%, the graph shows three clusters of patient discharge, "Normal Discharge", "Discharge after observation or voluntary or specialist" and "Death or Hospitalization". The first cluster is characterized by white triage code, the second cluster is mainly characterized by green and yellow codes and the last is characterized by red and yellow codes



specialist allergy assessment after being discharged. Only a small number of the patients, 172 patients, corresponding to about a third of those sent to a specialist, were registered in the database of the Piedmont Regional Hospital Network for Allergic Diseases by the specialist services at the Maggiore Hospital in Novara. Of these 34 were "yellow codes" and 3 were "red codes". During the period considered the most frequently recurring diagnoses for all the groups of patients were hives (34.3%) and asthma (23.4%), respectively; the great majority were mild cases. The number of "food allergy" cases is consistent: 2-3% of real, non-pollen related food allergies reported in the general population (12,13,27).

Our data show a probable under-estimation of the number of cases of anaphylaxis registered in Emergency Department while the number of deaths is higher than that expected by the various epidemiological studies (34,35). It is therefore presumed that some cases of anaphylaxis have not reached the hospital and that some sudden deaths in the territory have been attributed to other causes.

On the other hand 2 cases of anaphylaxis in 2007 were classified as "green code", one discharged as "voluntary discharge" and the other as "normal discharge".

An important fact is that "triage" screening by the nursing staff is not focused on coding allergic reactions, but because of staff awareness, an appropriate evaluation of severity was made in most of the cases treated.

The more severe cases were always properly coded: subjects who were "yellow" and "red" code required intensive care, either temporarily or during normal hospitalization. A total of 83/362 "yellow codes" and 51/71 "red codes" were hospitalized.

This is confirmed by the outcomes: most of "white code" and "green code" admissions were discharged after treatment (97,8% of the "white" code admissions and 68,3% of the "green" code admissions). However, the number of subjects who were referred to a specialist practice is too small compared to the need for making a proper diagno-

sis, which is vital to prevent subsequent relapses. In fact, the "white" code category had numerous diagnoses that deserved further investigation. Bronchial asthma is a chronic disease and even when assessed as being mild its evolution should not be overlooked (28). In the same way, hives tend to return and/or persist over time: cases of acute hives are often an expression of sensitivity to some allergen that can be avoided while exacerbated/chronic hives subjects, if not adequately treated, return several times to the E.D. for observation. Adverse reactions to drugs lead to a "label" being placed on the patient that influences the patient's behavior, the subsequent work of physicians and other health workers while the evolution of hymenoptera sting symptoms is not readily foreseeable without further investigations.

In the same way, a more detailed description of the diagnosis would be advisable in order to avoid the use of very general terms which could invalidate the collection of the epidemiological data needed to improve emergency hospital services.

In fact, by generically using the definitions "allergic reaction" or "insect sting-induced dermatitis" or "dermatitis" for a number of diagnoses which account for 18.5% of all admissions is impossible to understand really the overall impact of allergies on the work of an E.D. and, as a result, to quantify the resources that are required to deal with specific matters.

This information shows the need to improve the emergency clinical approach to allergic diseases and to plan the subsequent management of patients based on a medium and long term diagnosis/therapy. Especially for cases of anaphylaxis, it is essential to provide proper information on prevention and on how to deal with anaphylaxis and self-injectable epinephrine outside the hospital (28-30).

This can also be a source of useful information on how to improve the overall response to the health needs of allergic subjects, for example by comparing the data with that of other similar facilities in other areas, in order to determine how the presence of specialist centers can prevent/reduce suspected allergic acute events (11).

It would also be useful to create a greater sense of awareness among health workers when addressing patients to the appropriate specialists for the diagnosed disease, after receiving emergency treatment (26).

The epidemiological analysis of these cases is important because there is a link with the exposition of the environmental type.

The "acceptance" of the allergic subject allows the related cause – effect of the allergen/symptoms to be clarified and the set of information tools and therapies that can prevent and/or treat exacerbation to be prepared (25,32).

Dedicated it tools can now be used to print automatically information materials and guidelines for patients, by automatically linking the various print functions, keywords in the descriptive diagnosis and triage severity.

Emergency diagnosis and treatment can be performed by planning a standard in-house procedure to create more awareness of, and to train medical and non-medical staff.

The goal is to obtain a consistent diagnostic-therapeutic response by defining both the times and methods for hospital observation and by suggesting appropriate treatment at home and with priority access to specialist allergy centers.

It is just as important to provide users with information that is clear, logical and consistent with real clinical needs: often different departments of the same hospital give contradictory information.

The Emergency Department is currently a "brochure" that presents the hospital, and using the information on its activity to confirm that the system is working efficiently and to suggest further improvements is essential in guaranteeing that resources are put to the best scientific and clinical use.

References

1. Schoenwetter WF, Dupclay L Jr, Appajosyula S, Botteman MF, Pashos CL. Economic impact and quality-of-life burden of allergic rhinitis. Curr Med Res Opin. 2004;20:305-17.
2. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, Williams H; ISAAC Phase Three Study Group. 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.
3. Lundback B. Epidemiology of rhinitis and asthma. Clinical and Experimental Allergy. 1998; 2: 3-10.
4. Beasley R, Crane J, Lai CK, Pearce N. Prevalence and etiology of asthma. J Allergy Clin Immunol. 2000;105:S466-72.
5. Malone DC, Lawson KA, Smith DH, et al. A cost of illness study of allergic rhinitis in the United States. Journal Allergy and Clinical Immunology 1997; 99:22-27.
6. Babey SH, Meng YY, Brown ER, Hastert TA. Nearly six million Californians suffer from asthma symptoms or asthma-like breathing problems. Policy Brief UCLA Cent Health Policy Res. 2006 Oct;(PB2006-5):1-7.
7. Ciprandi G, Vizzaccaro A, Cirillo I, Crimi P, Canonica GW. Increase of Asthma and Allergic Rhinitis Prevalence in Young Italian Men. Internal Archives Allergy and Immunology, 1996:111, 279-83.
8. Berto P, Zanferrari G: Studi di cost-of-illness dell'asma: una re-

view della letteratura internazionale 1990-1995. Quaderni di Economia e Sanità, 1/97, 1997.
9. Leynaert B, Bousquet J, Neukirch C, Liard R, Neukirch F. Perennial rhinitis: an independent risk factor for asthma in nonatopic subjects: results from the European community respiratory health survey. Eur Ann Allergy Clin Immunol. 1999; 301-4.
10. Galassi C. Changes in prevalence of asthma and allergies among children and adolescents in Italy: 1994-2002. Pediatrics 2006; 117:34-42.
11. Allergy White Paper 1997.
12. Sicherer SH. Food allergy. Lancet. 2002;360:701-10.
13. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol. 2006 Feb; 117 (2 Suppl Mini-Primer): S470-5.
14. Demoly P, Bousquet J.:Epidemiology of drug allergy. Curr Opin Allergy Clin Immunol. 2001 Aug; 1 (4): 305-10.
15. Demoly P. Bousquet J. Drug allergy diagnosis work up. Allergy 2002; 57 Suppl 72: 37-40.
16. Avery NJ, King RM, Knight S, Hourihane JO. Assessment of quality of life in children with peanut allergy. Pediatr Allergy Immunol 2003;14:378-82.
17. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reaction to food. J Allergy Clin Immunol 2001;107:191-3.
18. Bohlke K, Davis RL, DeStefano F, Marcy SM, Brown MM, Thompson RS. Epidemiology of anaphylaxis among children and adolescents enrolled in a health maintenance organisation – J Allergy Clin Immunol. 2004; 113: 536-542.
19. Sampson HA, et al. Symposium on the Definition and Management of Anaphylaxis: Summary report J Allergy Clin Immunol. 2005; 115:584-91.
20. Sampson HA, et al. Second symposium on the definition and management of anaphylaxis: summary report--Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol. 2006 Feb;117(2):391-7.
21. Brown AF, MCKinnon D, Chu K. Emergency department anaphylaxis: A review of 142 patients in a single year. J Allergy Clin. Immunol. 2001; 108: 861 – 806.
22. Pastorello EA, Rivolta F, Bianchi M, Mauro M. Pravettoni V. Incidence of anaphylaxis in the emergency department og a general hospital in Milan. J Chromatogr B Biomed Sci Appl. 2001 May 25; 756 (1-2): 11-7.
23. Cadario G, Galimberti M, Rolla G. Emergenze allergologiche ed Anafilassi: diagnosi precoce e cenni di trattamento - Not. Allergologico 2003; 22 (3-4): 127-132 (www.lofarma.it 14/05/08)
24. Galimberti M, Maspoli M, Cadario G. La rete regionale di allergologia e l'Osservatorio per le gravi reazioni allergiche - Not. Allergologico 2002; 21 (4): 206 –9 (www.lofarma.it 14/05/08).
25. Abstract of the American Academy of Allergy Asthma and Immunology (AAAAI) 60th annual meeting. March 19-23, 2004. San Francisco, California, USA. J Allergy Clin Immunol. 2004 Feb; 113 (2 Suppl): S25-438.
26. Branco Ferreira M, Rodriguez Alves R. Are general practitioners alert to anaphylaxis diagnosis and treatment? Eur Ann Allergy Clin Immunol 2006; 38: (3) 83-6 .
27. Ortolani C, Bruijnzeel-Koomen C, Bengtsson U, Bindlev-Jensen C, Björksten B, Host A, Ispano M, Jarish R, Madsen C, Nekam K, Paganelli R, Poulsen LK, Wuthrich B. Controversial aspects of adverse reactions to food. Allergy 54: 27-45, 1999 .
28. Linee guida ARIA 2006.
29. Sicherer SH. Quandaries in prescribing an emergency action plan and self-injectable epinephrine for first- aid management of anaphylaxis in the community. J Allergy Clin Immunol 2005;115:575-83.
30. Simons FER. Anaphylaxis killer allergy: Long-term management in the community. J Allergy Clin Immunol 2006;117:367-77.
31. Muller U, Mosbech H. Position Paper: Immunotherapy with Hymenoptera venoms. EAACI. Allergy 1993;48:36-46.
32. Sampson HA. Anaphylaxis and emergency treatment. Pediatrics 2003;111:1601-8.
33. Sicherer SH, Simons FER and the Section on Allergy and Immunology. Self-injectable Epinephrine for First- Aid Management of Anaphylaxis. Pediatrics 2007; 119:638-46.
34. Bohlke K, Davis RL, DeStefano F, et al. Epidemiology of anaphylaxis among children and adolescent enrolled in a health maintenance organization. J Allergy Clin Immunol 2004;113:536-42.
35. Yocum MW, Butterfield JH, Klein JS, et all. Epidemiology of anaphylaxis in Olmstead County: a population study - J. Allergy Clin. Immunol. 1999; 104: 452 – 6.
36. Newcombe RG, Altman DG. Proportions and their differences. In: Altman DG, Machin D, Bryant TN, Gardner MJ. eds. Statistics With Confidence, 2nd edn. London BMJ Books, 2000:45-55.
37. Lebart L, Morineau A, Peiron M. Statistique exploratoire multidimensionelle, Dunod, Parigi, 1995.

D. Machado, B. Tavares, G. Loureiro, B. Conde, J. Cemlyn-Jones, G. Calado, N. Sousa, C. Chieira, C. Pereira

Body mass index and airway hyper-responsiveness in individuals without respiratory disease

[1]Immunoallergology Department. Coimbra University Hospital. Coimbra. Portugal
[2]Immunoallergology Department. Diagnosis Center. Covilhã. Portugal

Key words

Airway hyperresponsiveness, body mass index; lung volumes, obesity, pulmonary function testing

Summary

Background. *Overweight and obesity are major health issues in Western societies. They are related with a higher risk of different co-morbidities but their relationship with airway hyperresponsiveness (AHR) is still under discussion. Nevertheless, they are related to higher severity in asthma and other respiratory diseases. The aim of the study was to analyze the AHR in individuals with normal lung function without respiratory disorders, according to body mass index (BMI) calculation.* ***Methods.*** *We performed clinical observation and basal lung function tests (LFT) in 595 consecutive individuals in order to exclude respiratory disease. 377 individuals fulfilled the criteria of normal values according international guidelines. They were submitted to standardized treadmill exercise test followed by bronchodilator test. FVC, FEV1, FEF 25/75, RV and Raw were obtained at different conditions according to BMI groups (I: lean; II: normal; III: overweight; IV: obese).* ***Results.*** *55.2% of the sample was overweight or obese, and a significant relationship was found with female gender and older ages ($p=0.0046$ and $p<0.0001$ respectively). The positive response to exercise test or bronchodilator β2 agonists was not significantly frequent compared with the other groups. In obese individuals the exercise markedly reduced basal Raw and increased FEF 25/75. Lean individuals showed higher basal values of RV that was reduced upon exercise. Response to β2 agonists showed no differences according to weight biotypes.* ***Conclusion.*** *BMI hampers lung function in normal individuals, and seems not to be related to AHR. Regular exercise should be encouraged in overweight and obese individuals, since it increases their bronchial permeability as shown in lower frequency of positive exercise tests. The same is advisable for lean individuals for different reasons. Their increased basal RV and Raw improve upon exercise. Despite overweight and obesity are being related to a low-grade of basal systemic inflammation, there was no association with a higher basal bronchial hyperresponsiveness in these individuals.*

Introduction

Many studies have shown that there is a connection between increased BMI and asthma (1,2). With bronchial asthma being defined by GINA (Global Initiative for Asthma) as a chronic inflammation of the airways (3) and obesity as a pre-inflammatory condition (4), there has been a special interest in examining how the two are related. Additionally, reported incidence of asthma and obesity has increased in several countries (5-12) and the fact is that adult patients with asthma tend to be more obese than those without. In the last few years asthma has in-

creased in both children and adults (11), with incidence rates growing as societies become more westernized (11,12).

Obesity is a public health issue, especially associated to Western countries. In most European countries its rates have increased around 10 to 40% over the last few decades (8). In 2004, Lobstein and partners, in a study based on data from european countries, showed that the highest percentage of overweight children (aged between 7 and 10) occurred in the Mediterranean (10). According to International Obesity Task Force data (2003-2005, related to people aged between 18 and 64), in Portugal the percentage of overweight people was 39.4% (45.2% for men and 34.4% for women) and the percentage of obese people was 14.2% (15% for men and 13.4% for women) (9).

Obesity is generally considered a cardiovascular risk factor, but obesity also has an adverse impact on the respiratory system, namely as a result of changes in lung function, respiratory mechanisms, muscular strength and resistance, air exchange, and breathing control (6). Various studies conducted with both children and adults point to the correlation between asthma and obesity, showing that obesity precedes asthma, the relative risk of developing asthma increases with BMI, and obesity makes asthma difficult to control or increases its seriousness (13,14).

The possible processes that explain the correlation between asthma and obesity are in summary, the following: mechanical effects of obesity, systemic and chronic inflammation, energy regulating hormones, co-morbidity and common etiology (13).

In terms of the mechanical effects of obesity, the decrease in the lung-thorax compliance is one of the primary consequences of obesity (6,14). Associated to the change in chest wall elasticity, comes the decrease in residual functional capacity, residual exhalation volume and the width of the airways, the restriction in deep breathing, a marked contraction of the smooth respiratory muscle and of the affected physiological bronchodilation mechanism (13,15,16). These changes lead to a reduction in lung capacity, an increase in bronchial hyperresponsiveness and lung blood levels and a ventilation-perfusion mismatch (13,14).

We know that obesity is a low-level systemic inflammation condition. Obese people's adipose tissues include various pro-inflammatory molecules such as cytokines, chemokines, complementary proteins and acute phase proteins (adipokines) (13,17). There appears to be a significant correlation between the immune function of the adipocytes and the T-lymphoctye and macrophage function, mainly in the creation of inflammatory cytokines (17). We also highlight the importance of the energy regulating hormones in relating asthma to obesity, especially in terms of leptin and adiponectin (13). Leptin is important in appetite-regulation, inducing a feeling of satisfaction or fullness and an increase in metabolic rate. This hormone occurs in markedly higher levels in obese people (13,16). Recent studies have suggested that leptin increases the hyperresponsiveness of the airways, albeit through a mechanism that is independent of Th2 inflammation 13,16,18. Adiponectin, an insulin regulating hormone, occurs at reduced levels in obesity. This hormone has key anti-inflammatory functions in obesity, as it inhibits the production of pro-inflammatory cytokines and increases the production of IL-10 and the IL-1 inhibitor (19). Increasingly, there is a suggestion that adiponectin significantly reduces bronchial hyperresponsiveness, inflammation of the airways and the occurrence of Th2 cells in lungs (20-22).

Most of the works published on the effect of BMI on respiratory function has been based on asthmatic patients. There are very few studies based on people without a respiratory disease or severe breathing problems. In fact it is a point of discussion whether obesity is a risk factor for AHR.

The aim of the study was to analyse the AHR in individuals with normal breathing function and without respiratory disorders or illness, according different phenotypes obtained from the BMI calculation.

Material and methods

This was a prospective study conducted between January to December 2006, obtained by the analysis of 595 consecutive both sex individuals sent by medical general practitioner (GP) in order to exclude bronchial and respiratory disease. All of them were submitted to a pletismographic test (MasterLab Jaeger), undertaken by the same cardio-respiratory technician. The following protocol was implemented:

1. Baseline measurements of dynamic volumes, static volumes and airways resistance, as the best of two measures.
2. Treadmill exercise test (Exer), while breathing ambient air (20ºC) with a nose clip to ensure mouth breathing, and cardio monitoring. In order to achieve approximately 80% of the maximum predicted heart rate (220-age in years) after a 1 minute warm-up at a lower work

rate, the patients performed a near maximal constant load exercise for 6 minutes in a treadmill. At least two acceptable FEV1 values were obtained at 1 and 5 minutes after cessation of exercise and the lowest FEV1 value was selected to calculate the fall from baseline by the following equation: % fall in FEV1 = (pre-exercise FEV1 - lowest FEV1 post-exercise) / pre-exercise FEV1 x 100%. Those with a fall in FEV1 ≥15% were considered positive for exercise test.

3. Bronchodilator test (BD), administering an inhaled short acting β2 inhibitor (100 μg of albuterol) through a spacer; the lung function was re-assessed 15 minutes later. It was considered a positive bronchodilator response an increase in FEV1 and/or FVC ≥12% of control.

The procedures and the technique were according to ATS/ERS Task Force criteria, as well as the interpretation of the results (23-26). The following lung function parameters were analyzed: forced vital capacity (FVC), forced expiratory volume at 1 second (FEV1), mean forced expiratory flow between 25% and 75% of FVC (FEF 25/75), residual volume (RV) and airway resistance (Raw).

All the non-smokers individuals with normal basal lung function tests (FEV1 and FVC ≥80%) and that had no clinical evidence of respiratory disease or other relevant illnesses were included on the study.

This sample was divided into 4 groups, according to the International Classification of adult BMI – WHO (5) (Tab. 1).

The difference between gender and age groups was analysed using the χ^2 test. Kruskal-Wallis test was used to establish the statistical significance of each respiratory factor and Mann-Whitney test was used to assess the statistical differences within each BMI group. To study the impact of physical exercise and bronchial dilation on lung function, the Wilcoxon Signed Ranks test was used. The statistical assessment was performed using the SPSS 15.0® program (2006 SPSS Inc, Chicago, Ill, USA). $p<0.05$ was considered as the statistical relevance standard.

Table 1 - BMI groups according to WHO classification

Group	Classification	BMI(kg/m^2)
I	Underweight	<18.50
II	Normal range	18.50 – 24.99
III	Overweight	25.00 – 29,9
IV	Obese	≥30.00

Results

Of the 595 people who were tested, 377 met the criteria defined (238 were women and 139 were men), non-smokers, all without illness and with normal respiratory function base levels. The average age was 41.33±17.92. The average BMI was 25.94±4.71.

Table 2 shows the demographic data and lung function test results according to BMI groups. The results of lung function parameters in the three steps of evaluation are shown in figure 1.

55.2% of the individuals were overweight or obese (Group III and IV), and a significant relationship with female gender ($p=0.0046$) and older ages was found ($p<0.0001$).

Group II had the highest FVC basal value. This parameter increased with exercise in almost all the sample, albeit with greater significance in Group I. The bronchodilator test improved FVC in all groups but mainly in Groups II, III and IV.

FEV1 deteriorated with exercise in Groups II and III. In Group I, this parameter improved but not significantly. The bronchodilator test improved FEV1 in all Groups, but more noticeable in Groups II, III and IV.

In terms of the distal air flow, FEF 25/75 average basal values were highest in Group I. Exercise improved FEF 25/75 for individuals in Groups III and IV, mainly in the latter. Individuals in Groups I and II (underweight or normal) and who are as previously mentioned younger, showed marginally worse levels with exercise.

In lean individuals basal RV values were higher compared to the other groups. They were slightly reduced by exercise in opposite to obese individuals although without statistical significance.

Basal airway resistance mean values were higher in group IV, and markedly reduced by exercise.

Positive exercise challenge test was uncommon among the total sample, with the overweight and obese individuals showing curiously the lowest frequencies (Fig. 2). Responsiveness to bronchodilator was presented at higher rates, but without significant differences between groups.

Discussion

This study differs from the previous ones, given that the study population included individuals who had normal respiratory function according to the criteria established by ATS/ERS Task Force (23,24), and had no respiratory disease.

Table 2 - Summary table with main lung function test results by BMI grouping. Values are shown as average percentage with standard deviations

	Group I	Group II	Group III	Group IV
N	16	153	128	80
Female	8	87	84	59
Male	8	66	44	21
Average age	10.56±4,89	34.62±16,66	47.84±15,39	49.9±13,66
FVC	86.61±6.03	98.19±11.64	96.77±12.56	96.74±11.92
FVC – exercise	100.36±7.44	98.43±7.18	99.45±6.67	98.75±6.25
FVC – BD	102.80±7.19	102.11±6.91	103.18±7.70	103.23±7.05
FEV1	100.43±8.37	106.56±11.02	104.77±11.98	104.36±13.27
FEV1 – exercise	100.75±6.38	99.95±5.69	101.39±5.28	101.71±6.16
FEV1 – BD	102.34±5.17	103.07±5.59	103.33±6.01	104.61±6.59
FEF 25/75	119.89±23.98	110.42±29.49	103.32±28.00	102.06±24.40
FEF 25/75 – exercise	108.40±12.94	105.77±16.51	107.68±16.08	113.03±20.85
FEF 25/75 – BD	106.29±15.37	107.95±18.03	105.84±21.23	110.88±20.31
RV	116.61±22.26	100.64±29.62	93.68±25.35	90.11±30.53
RV – exercise	98.57±41.43	112.69±44.79	101.17±33.31	111.93±75.59
RV – BD	92.33±21.82	106.32±48.64	98.54±27.48	103.09±43.24
RAW	135.85±51.39	93.51±43.59	125.00±68.35	146.03±84.09
RAW – exercise	125.49±32.89	108.25±39.81	102.22±36.56	96.21±36.98
RAW – BD	103.88±20.41	96.37±30.84	94.37±31.82	89.84±32.29

In order to measure airways reactivity, there are several tests that can be performed; the methacoline and exercise challenging tests are the most widely used, but the first one is better established (26). Although challenge tests with methacholine are more sensitive in the diagnosis of AHR than tests with exercise, the last ones are more specific and may reflect more directly the ongoing airway inflammation (27). Also, there are patients with mild bronchial AHR to methacoline who have negative exercise challenges and others who have positive exercise challenges but negative methacholine challenges; this can be indicative of different mechanisms involved in AHR (27). We chose the exercise challenge test because it is the most physiological and reflect a natural trigger to AHR; also, to this date there are very few studies that investigated exercise induced bronchospasm.

Our results suggest that BMI affects lung function in people without a respiratory disorder. This agrees with the data of several other studies, in which it was found that the lung capacity varied inversely with the body weight (2, 28-31). The changes in resistance, as a function of BMI, are also in agreement with these studies, suggesting that obese people have higher airways resistance (32-34). One curious found in our study is that underweight people also showed higher base level resistance. These data could be explained by the fact that the BMI groups were defined by different criteria, depending on the study, and most of the samples had mainly obese people.

In our study the different BMI groups did not appear to have more frequently AHR despite having normal respiratory function according to the ATS/ERS criteria (23,24). Obesity was not linked to an increase in AHR. Therefore, we conclude that although there is no clear rise in the frequency of hyperresponsiveness, we suspect that depending on the clinical conditions which led to obese patients developing asthma, this would be more serious, as a result of the bronchial inflammation induced by the increased synthesis of leptin by the adipocytes and the systemic inflammation caused by obesity, as a result of increased TNF-α, IL-6 and sIL-6R levels (6,13,17), as suggested by a study published by our group (35). This can also explain the higher base level of airways resistance in obese and also overweight people. These groups of individuals showed less bronchial obstruction following exercise and the best response to bronchodilators. Obese and overweight subjects tend to have decreased pulmonary

Figure 1 - Effects of BMI on FVC, FEV1, FEF25/75, RV and resistance (RAW), according to lung function tests.The horizontal lines are the between-group comparisons from Wilcoxon Signed Ranks and Mann-Whitney tests.The vertical lines are the error bars (2 SD)

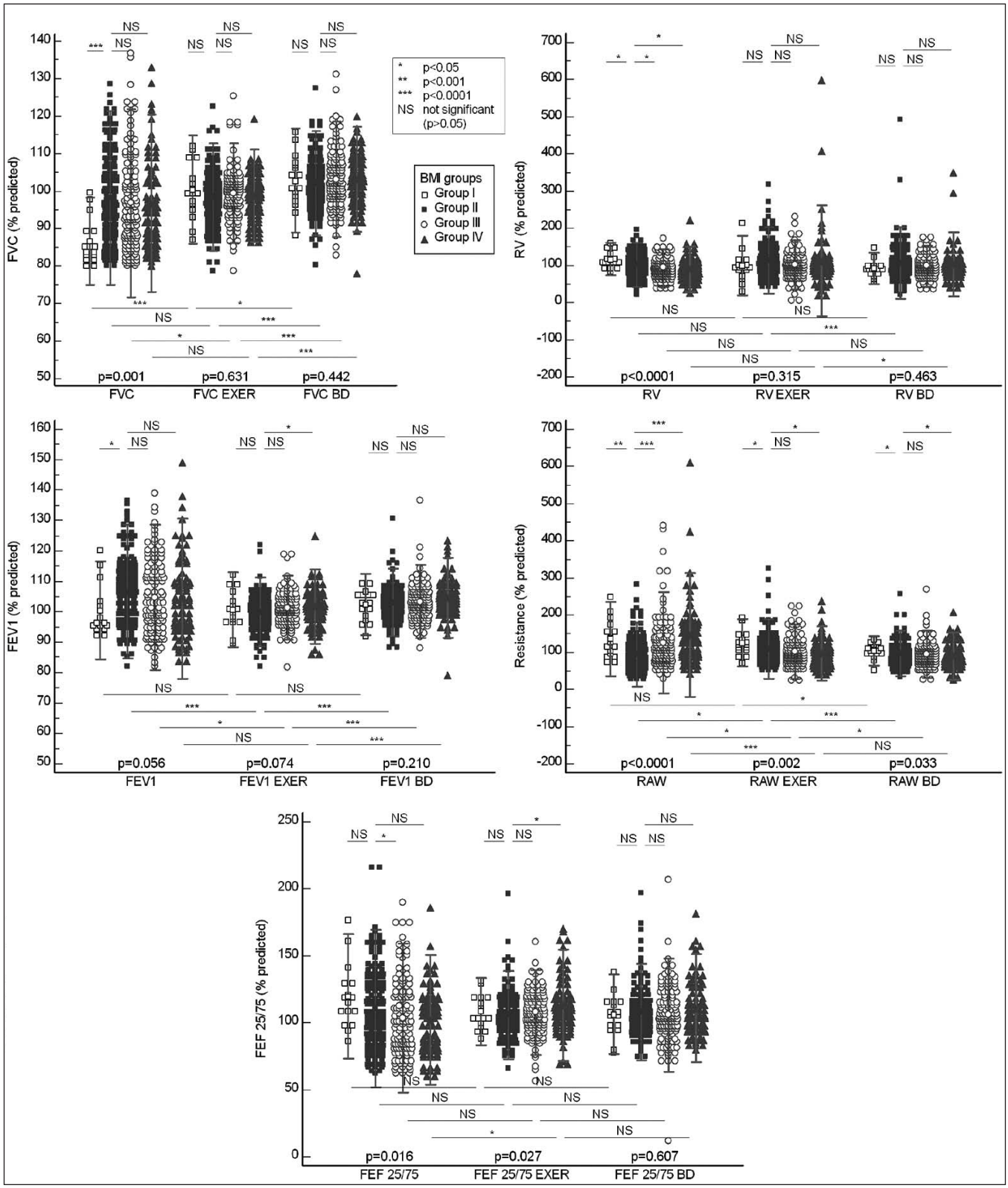
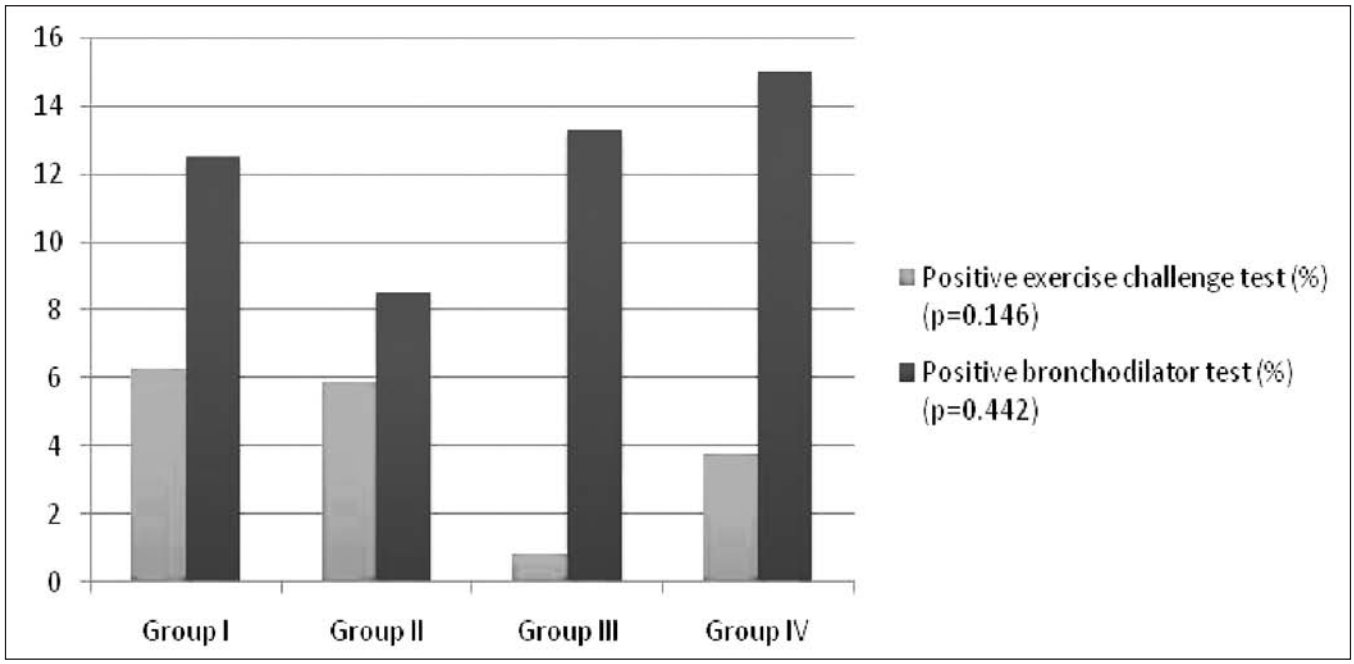


Figure 2 – Exercise challenge and positive bronchodilator tests (as a %), distributed by BMI Groups



volumes and less thoracic wall distensibility (36); they have a more sedentary life and are not used to exercise, so with physical activity they have to exercise muscles, otherwise not employed, and better coordinate their respiratory movements, which can explain a better bronchomotricity.

The role of obesity on AHR is controversial, as arise from the analysis of several studies. In 2006, a study undertaken by Johnston and cols concluded that obese rats showed increased lung response to ozone, and that the inhalation of this gas increased resistance, hyperresponsiveness and inflammation of the airways (37). These results were then reproduced in human, where some studies showed higher increase in AHR and a reduction in lung function response to ozone in overweight as opposed to underweight individuals (38,39). Chinn and cols, 2002, showed that there was a significant correlation between bronchial hyperresponsiveness to methacoline and BMI, but only in men (40). Another study published in 2002, conducted in men participating in the Normative Aging Study, associated both a low BMI and a high BMI with the development of AHR to methacholine (41). Although our study did not find a relation between BMI and AHR, underweight and obese individuals had the lowest basal FEV1 and FCV levels and the highest basal resistance levels, and exercise improved the general lung function parameters. The differences between the studies referred before and our study can be explained by the less sensitivity and more specificity of exercise challenge tests in the diagnosis of AHR (27), as already explained. Also, we have highlighted before that obese people do not have a regular physical activity, so exercise can bring a better respiratory coordination and muscles utilization, facts that can explain the respiratory improvement with exercise in these individuals. Other studies showed no correlation between BMI and AHR. Schachtern and partners in 2001 did not find any connection between serious obesity and AHR to histamine challenge, despite obesity being a factor for asthma and wheezing (42). Weight loss did not lead to a reduction in bronchial hyperresponsiveness to methacoline in a group of obese women (43).

Various studies have attempted to establish the relationship between obesity and asthma, and have reached diverse conclusions. In 2005 a review of several studies concluded that adult asthma patients are more obese than those without asthma, but in children and adolescents this correlation is less marked (1). The prevalence of asthma is higher in obese individuals, as obesity underlines a worse clinical history (16). Longitudinal studies show that obesity precedes asthma and that the risk of asthma amplifies with increasing BMI (44,45). Obesity is related to

a worse clinical history and makes the asthma more difficult to control (13,43). One interesting study was conducted with perimenopausal women, which established that underweight and obese women showed the highest risk of having impaired lung function and of developing asthma. In underweight women the explanation was that the fatty tissue produces less oestrogen, while in obese women the explanation arises from insulin-resistance, as this is a pro-inflammatory factor (46).

Our results stress that BMI seems not be related to higher risk of AHR, namely in overweight and obese individuals. They must be asked for regular exercise, because they increase their bronchial permeability besides the lower positivity to exercise test. Lean individuals should also be asked for regular exercise, because they decrease the values of basal RV and Raw. Despite the lower frequency of positive broncoresponsiveness tests in our study among overweight and obese individuals we strong believe that if asthma occurs in those, it definitely contributes to a severe clinical phenotype, developed on a previous low-grade inflammatory state already described.

References

1. Ford ES. The epidemiology of obesity and asthma. J Allergy Clin Immunol 2005;115:897-909.
2. Jones RL, Nzekwu. The effects of Body Mass Index on Lung Volumes. Chest 2006;130:827-33.
3. Bousquet J, Clark TJ, Hurd S, et al. GINA guidelines on asthma and beyond. Allergy. 2007;62:102-12.
4. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005;115:911-9.
5. Obesity: preventing and managing the global epidemic. WHO Technical Report Series 894. WHO Consultation on obesity. Geneva (Switzerland); 2000.
6. Veale D, Rabec C, Labaan JP. Respiratory complications of obesity. Breathe 2008;4:211-23
7. Charles MA, Basdevant A, Eschwege E. Prevalence of obesity in adults in France: the situation in 2000 established from the OBEPI Study. Ann Endocrinol (Paris) 2002;63:154-8.
8. Lobstein T, Millstone E; PorGrow Research Team. Context for the PorGrow study: Europe's obesity crisis. Obes Rev 2007;8 (Suppl 2):7-16.
9. James WP. The epidemiology of obesity: the size of the problem. J Intern Med 2008;263:336-52.
10. Lobstein T, Baur L, Uauy R. IASO International Obesity TaskForce. Obesity in children and young people: a crisis in public health. Obesity Reviews 2004;5 Suppl 1:4-104.
11. Masoli M, Fabian D, Holt Shaun, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee Report. Allergy 2004;59:469-78.
12. Douwes J, Pearce N. Asthma and the westernization "package". Int J Epidemiol 2002;31:1098–102.
13. Shore SA. Obesity and Asthma: possible mechanisms. J Allergy Clin Immunol 2008;121:1087-93.
14. Beuther DA, Weiss ST, Sutherland ER. Obesity and asthma. Am J Respir Crit Care Med 2006;174:112-9.
15. Sood A. Does obesity weigh heavily on the health of the human airway? J Allergy Clin Immunol 2005;115:921-4.
16. Shore SA, Fredberg JJ. Obesity, smooth muscle, and AHR. J Allergy Clin Immunol 2005;115:925-7.
17. Shore SA. Obesity and asthma: lessons from animal models. J Appl Physiol 2007;102: 516-28.
18. Williams AS, Leung SY, Nath P, Khorasani NM, Bhavsar P, Issa R, et al. Role of TLR2, TLR4, and MyD88 in murine ozone-induced AHR and neutrophilia. J Appl Physiol 2007;103:1189-95.
19. Fantuzzi G. Adiponectin and inflammation: consensus and controversy. J Allergy Clin Immunol 2008;121:326-30.
20. Shore SA, Terry RD, Flynt L, Xu A, Hug C. Adiponectin attenuates allergen-induced airway inflammation and hyperresponsiveness in mice. J Allergy Clin Immunol 2006;118:389-95.
21. Okamoto Y, Folco EJ, Minami M, Wara AK, Feinberg MW, Sukhova GK, et al. Adiponectin inhibits the production of CXC receptor chemokine ligands in macrophages and reduces T-lymphocyte recruitment in atherogenesis. Circ Res 2008;102:218-25.
22. Alberti L, Gilardini L, Girola A, Moro M, Cavagnini F, Invitti C. Adiponectin receptors gene expression in lymphocytes of obese and anorexic patients. Diabetes Obes Metab 2007;9:344-9.
23. R. Pellegrino, G. Viegi, V. Brusasco, et. al. Interpretative strategies for lung function tests. Eur Respir J 2005;26:948–68.
24. American Thoracic Society. Lung Function Testing: Selection of Reference Values and Interpretative Strategies. Am Rev Respir Dis 1991;144:1202–18.
25. Roca J, Whipp BJ, Agustí AGN, et al. ERS Task Force on Standardization of Clinical Exercise Testing. Clinical exercise testing with reference to lung diseases: indications, standardization and interpretation strategies. Eur Respir J 1997;10:2662–89.
26. Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing-1999. Official Statement of the American Thoracic Society. Am J Respir Crit Care Med 2000;161:309–29.
27. Joos GF et al. Indirect airway challenges. Eur Respir J 2003;21:1050–68.
28. Sahebjami H. Dyspnea in obese healthy men. Chest 1998;114:1373–7.
29. Ray CS, Sue DY, Bray G, et al. Effects of obesity on respiratory function. Am Rev Respir Dis 1983;128:501–6.
30. Jenkins SC, Moxham J. The effects of mild obesity on lung function. Respir Med 1991;85:309–11.
31. Rubinstein I, Zamel N, DuBarry L, et al. Airflow limitation in morbidly obese, nonsmoking men. Ann Intern Med 1990;112:828–32.
32. Zerah F, Harf A, Perlemuter L, et al. Effects of obesity on respiratory resistance. Chest 1993; 103:1470–6.
33. Ferretti A, Giampiccolo P, Cavalli A, et al. Expiratory flow limitation and orthopnea in massively obese subjects. Chest 2001; 119:1401–8.
34. Watson RA, Pride NB. Postural changes in lung volumes and respiratory resistance in subjects with obesity. J Appl Physiol 2005; 98:512–7.
35. B Tavares, C Pereira, C Ribeiro, D Machado, G Loureiro, C Chieira. Obesity and severity of asthma. Allergy 2007; 62 (supl 83):201.

36. del Rio-Navarro BE, Cisneros-Rivero MG, Berber-Eslava A, et al. Exercise induced bronchospasm in asthmatic and non-asthmatic obese children. Allergol et Immunopathol 2000;28:5–11.
37. Johnston RA, Theman TA, Shore SA. Augmented responses to ozone in obese carboxypeptidase E-deficient mice. Am J Physiol Regul Integr Comp Physiol 2006;290:R126–33.
38. Bennett WD, Hazucha MJ, Folinsbee LJ, Bromberg PA, Kissling GE, London SJ. Acute pulmonary function response to ozone in young adults as a function of body mass index. Inhal Toxicol 2007;19:1147-54.
39. Alexeeff SE, Litonjua AA, Suh H, Sparrow D, Vokonas PS, Schwartz J. Ozone exposure and lung function: effect modified by obesity and airways hyperresponsiveness in the VA normative aging study. Chest 2007;132:1890-7.
40. Chinn S, Jarvis D, Burney P, European Community Respiratory Health Survey. Relation of bronchial responsiveness to body mass index in the ECRHS. European Community Respiratory Health Survey. Thorax 2002;57: 1028–33.
41. Litonjua AA, Sparrow D, Celedon JC, DeMolles D, Weiss ST. Association of body mass index with the development of methacholine airway hyperresponsiveness in men: the Normative Aging Study. Thorax. 2002;57:581-5.
42. Schachter LM, Salome CM, Peat JK, Woolcock AJ. Obesity is a risk for asthma and wheeze but not AHR. Thorax 2001;56:4–8.
43. Aaron SD, Fergusson D, Dent R, Chen Y, Vandemheen KL, Dales RE. Effect of weight reduction on respiratory function and airway reactivity in obese women. Chest 2004;125:2046–52.
44. Camargo CA Jr, Weiss ST, Zhang S, Willett WC, Speizer FE. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. Arch Intern Med 1999;159:2582-8.
45. Guerra S, Wright AL, Morgan WJ, Sherrill DL, Holberg CJ, Martinez FD. Persistence of asthma symptoms during adolescence: role of obesity and age at onset of puberty. Am J Respir Crit Care Med 2004;170:78-85.
46. Real FG, Svanes C, Omenaas ER, et al. Lung function, respiratory symptoms, and the menopausal transition. J Allergy Clin Immunol 2008;121:72-80.

C. Lombardi[1], S. Gargioni[1], G.W. Canonica[2] , G. Passalacqua[2]

The nocebo effect during oral challenge in subjects with adverse drug reactions

[1] Pneumoallergology Unit, Sant'Orsola FBF Hospital, Brescia
[2] Allergy and Respiratory Diseases, Dept of Internal Medicine, University of Genoa

Key words

Drug challenge, adverse drug reaction, nocebo effect

Summary

Background: *Nocebo effect is the occurrence of troublesome symptoms after the administration of inert substances. It can be easily studied during blind oral challenges for drug reactions, which always involve the administration of a placebo.* ***Methods:*** *We collected data about nocebo effect in outpatients undergoing oral drug challenge. Patients with previous documented adverse reactions to drugs underwent an oral challenge with alternative drugs to identify the compounds that can be safely used. The challenges involved the administration of a placebo before the active drugs and were performed under medical supervision.* ***Results:*** *Four hundred and thirty-five patients (18-68 yrs, 68% female) underwent the oral drug challenge. Most of them (52%) had a previous reaction with antibiotics and non-steroidal antinflammatory drugs. The reported reactions were urticaria (and/or angioedema), respiratory complaints, generalized itching and non-specific symptoms. A nocebo effect was seen in 13 patients (3%), (10 female). The majority of the observed effects were subjective (malaise, itching, abdominal pain). No special demographic or clinical characteristic could be identified in the nocebo reactors. Ten/13 patients had an abnormal result at the hospital anxiety depression questionnaire.* ***Conclusion:*** *Nocebo effect was not negligible in this procedure, although less frequent than previously reported.*

Introduction

The "placebo effect" is defined as the beneficial action, based on patient's expectation, exerted by an inert substance on the symptoms of a disease. Of note, in recent years an increasing attention has been devoted to the power of sham medications in clinical practice (1, 2), and the use of placebo has been extensively discussed (3, 4). By contrary, the "nocebo effect" is the occurrence of troublesome symptoms after the administration of inert substances (5). The nocebo response is usually subjective (e.g. nausea, headache, itching, feelings of cold or warmth), but it may also be objective (e.g. rush, urticaria, vomiting, tachycardia, changes in blood pressure). Similarly to the placebo effect, the nocebo effect is influenced by several factors such as patient's expectation, previous experiences, setting, appearance of the drug.

In some cases of adverse reactions to drugs (ADR), the only reasonable way to manage the problem is to identify the alternative drugs which can be used safely. This is usually done by means of a single-dose or an incremental oral challenge with the alternative drug (6, 7). In this case, the blind use of a placebo is mandatory in order to rule out the possible psychosomatic reactions.

Patients with ADR undergoing an oral drug challenge represent an ideal model to study the nocebo effect. We prospectively evaluated the occurrence and characteristics of the nocebo effects in patients with previous adverse drug reactions, seen in a 5-year period at our Allergy Unit in an hospital setting.

Methods

Consecutive outpatients seen at our Allergy Unit (Department of Internal Medicine, Sant'Orsola Hospital, Brescia), undergoing an oral challenge with drugs were evaluated concerning the occurrence of nocebo effects. The patients with indication to the oral challenge were those with reported urticaria/angioedema, generalized itching, respiratory symptoms (cough, chest tightness, wheezing) or laryngeal oedema after the use of a given drug. Patients with a history of anaphylaxis, Stevens-Johnson syndrome or other severe skin reactions to drugs were not admitted to the challenge. Those patients with major systemic diseases (insulin-dependant diabetes, arrhythmias, severe uncontrolled asthma or systemic autoimmune diseases) were excluded as well.

The clinical ADR history was evaluated by trained allergists, based on the documentation from GPs or from emergency care units. ADRs had to have occurred within 24 hours from the intake of a single drug. In the case of subjective symptoms (e.g. itching, malaise, headache), the symptoms had to be present at least in two occasions, with the same drug and with the same time of onset.

All patients underwent these following procedures before the oral challenge test: detailed clinical history, physical exam, electrocardiogram, signed and informed consent, and they had to be symptom free since at least one week. A peripheral intravenous access was positioned, and arterial blood pressure and oxygen saturation were constantly monitored during the challenge. The challenges were performed under continuous medical supervision and with emergency care equipment immediately available. An Ethical Committee approval was not required as the procedure is considered part of the routine procedures used in the hospital.

The oral challenge involved the administration of one (or more) drug(s), different in structure from those suspected to have caused ADRs, irrespective of the mechanism. In fact, aim of the challenge is to identify for each patient a drug to be taken safely if needed (8). Drugs were given in capsules containing either different amounts of the active principle (between 1/10 and 1 of a therapeutic dose), or talcum. All capsules were packed by specialized personnel of the Internal Medicine Department. The challenges were single-blinded and the placebo always preceded the administration of the active drug(s), but the patients were not informed of the presence of the placebo in the procedure. Patients were observed for at least eight hours after each administration, and any possible problem/symptom was recorded by the attending physician.

The Hospital Anxiety and Depression (HAD)(9) questionnaire was administered to those patients who reacted to the administration of the placebo (nocebo effect). The questionnaire is a self-screening scale for depression and anxiety and is a reliable tool for detecting those states in the setting of an hospital outpatient clinic. The anxiety and depression subscales also measure the severity of the emotional disorder. The range of HAD is: 0-7 = normal; 8-10 = borderline; 11-21 = abnormal.

Results

Four hundred and thirty-five patients underwent the oral drug challenge between 2002 and 2007. Their demographic characteristics are summarized in table 1. Most of them (52%) had a previous reaction with antibiotics (38% beta-lactams), followed by non-steroidal antinflammatory drugs (NSAIDs) (41%), whereas a minority of patients had ADRs with diverse other drugs (e.g. anesthetic agents, chemotherapy agents, antispastics, glucocorticosteroids). The reported ADRs were urticaria (and/or angioedema) in 86% of patients, followed by respiratory complaints, generalized itching and other non-specific symptoms as tachycardia, headache and generic malaise (Table 2).

A nocebo effect (untoward reaction after placebo) was seen in 13 patients (3%), 10 of whose were female. The characteristics of the nocebo reactions are summarized in table 3. The majority of the observed effects were subjective (malaise, itching, abdominal pain), whereas in few occasion objective symptoms were reported. Placebo reactors did not differ from those who had no reaction concerning demographic and clinical characteristics (including the type of previous reaction and atopic status). In particular, the coexistence of atopic status (rhinoconjuctivitis, asthma,atopic dermatitis, food allergy and/or positivity of aeroallergens or food allergens) was low (2 cases;

Table 1 - Demographic and clinical data of the patients

Total number of patients	435
Age range	18-68 yrs
Mean age	39.7 yrs
Male/Female ratio	139/296 (F: 68%)
Patients reacting to placebo (%)	13 (3%)
M/F ratio reacting to placebo	3/10
Mean age of patients reacting to placebo	35.4 yrs

Table 2 - Clinical characteristics and frequency of the drug reactions reported in the clinical history (435 patients). Some patients had more than one symptom

Description	N	%
Urticaria, angioedema or both	374	86.1
Respiratory symptoms (rhinitis, wheeze, chest tightness, dyspnea, shortness of breath)	89	20.4
Laryngeal oedema	6	1.4
Generalized itching	55	12.6
Nonspecific (malaise, headache, tachycardia)	42	9.6

Table 3 - Clinical symptoms and signs of the reactions to placebo (most patients had multiple symptoms/signs at the same time)

Itching	9
Nausea	5
Abdominal pain	4
Headache	2
Dyspnea/cough	3
Tachycardia/bradycardia	5
Erythema/rash/urticaria	2
Gneralised malaise	6
Anxiety	8
Laryngeal obstruction subjective sensation	2
Eye vision alterations (not documented)	1

about 20%) and similar to the rate recorded in non-reactors (12/422). Also, there was no apparent correlation between the severity of the previous drug reaction and the onset of nocebo effect. In addition, we found that 3/13 patients (2 female) had a frankly abnormal result at the HAD questionnaire and 7/13 (6 female) had a borderline result.

Discussion

The effects of substances without pharmacological actions are well known in medical practice, and the placebo effect is a matter of fact (1, 10). The clinical aspects of the opposite phenomenon (nocebo effect) has been extensively considered as well, since also the nocebo effect may be of relevance in many clinical trials (11). Patients with previous adverse reaction to drugs are particularly susceptible to the nocebo effect, since they had experienced previous side effects and, more or less consciously, expect new troublesome reactions. The blind oral challenge with alternative drugs is a good model to study this effect. There are, in fact, some studies in literature on this topic (8, 12), consistently showing that some untoward reaction to placebo occurs in about one quarter of the patients.

Our data confirm some of the already described facts, such as the well-known higher prevalence of female (13) and the subjective nature of the nocebo effect. On the other hand, in our patients the rate of nocebo effect was quite lower (3%) than in other similar articles where the occurrence was reported up to 21% (8). This is difficult to explain, although some hypotheses can be suggested, involving a different empathy of the medical personnel, the cultural differences among the patients studied, or the influence of the medical environment. None of these hypotheses could be experimentally verified in this context. Another possible explanation of the aforementioned discrepancy is that in the present study those patients with more severe reactions (e.g. anaphylaxis or exfoliative dermatitis) were not admitted to challenge. It can be hypothesized that patients who experienced a very severe reaction are more prone to develop a nocebo effect, as a result of their expectation. Despite this speculation, there is in literature no correlation between the severity of the previous drug reaction and the occurrence of the nocebo effect (8, 12). Finally, another interesting observation of our survey was that the majority of patients with the nocebo effect, had an abnormal result to the hospital anxiety-depression questionnaire, this suggesting and confirming the relevant weight of the psycho-emotional situation of the subject in determining the clinical reaction to placebo.

References

1. Beecher HK. The powerful placebo. JAMA 1995; 159: 1602-06.
2. Turner JA, Deyo RA, Loeser JD, Von Korff M, Fordyce WE. The importance of placebo effects in pain treatment and research. JAMA 1994; 271. 1609-14.
3. Vickers AJ, de Craen AJ. Why use placebos in clinical trials? A narrative review of the methodological literature. J Clin Epidemiol 2000; 53: 157-61.
4. Kleijnen J, de Craen AJ, van Everdingen J, Krol L. Placebo effect in double blind clinical trials: a review of it interaction with medications. Lancet 1994; 344: 1347-9.
5. Keeley R. When are medication side effects due to the nocebo phenomenon? JAMA. 2002 May 15;287(19):2503; author reply 2503-4.

6. Bernstein IL, Gruchalla RS, Lerr ER, Nicklas RA, Dykewicz MS edts. Disease Management of Drug Hypersensitivity: A Practice Parameter. Ann Allergy Asthma Immunol 1999; 83: 667-71.
7. Aberer W, Bircher A, Romano A, Blanca M, Campi P, Fernandez J, Brockow K, Pichler WJ, Demoly P; European Network for Drug Allergy (ENDA); EAACI interest group on drug hypersensitivity. Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. Allergy 2003;58:854-63.
8. Passalacqua G, Milanese M, Mincarini M. et al. Single dose oral tolerance test with alternative compounds for the management of adverse reactions to drugs. Int Arch Allergy Immunol 2002; 129: 242-7.
9. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res. 2002; 52: 69-77.
10. Ernst E, Resch KL: Concept of true and perceived placebo effects. BJM 1995; 311: 551-53.
11. Barsky AJ, Saintfort R, Rogers MP, Bours JF. Nonspecific medication side effects and the nocebo phenomenon. JAMA 2002; 287: 622-7.
12. Liccardi G, Senna G, Russo M, Bonadonna P, Crivellaro M, Dama A, D'Amato M, D'Amato G, Canonica GW, Passalacqua G. Evaluation of the nocebo effect during oral challenge in patients with adverse drug reactions.J Investig Allergol Clin Immunol. 2004; 14:104-7.
13. Strohle A. Increased response to a putative panicogenic nocebo administration in female patients with panic disorder. J Psychiatr Res 2000; 34: 439-42.

R. Bernardini[1], S. Pecora[2], M. Milani[3], S.E. Burastero[3]

Natural rubber latex allergy in children: clinical and immunological effects of 3-years sublingual immunotherapy

[1] Paediatric Allergy and Pneumology Centre, Anna Meyer Children's Hospital, Florence, Italy;
[2] ALK-Abellò S.p.A., Milan, Italy; [3] San Raffaele Scientific Institute, Milan, Italy

Key words

Allergy, latex, sublingual immunotherapy, children

Summary

Background: *We previously demonstrated that one year of sublingual immunotherapy (SLIT) with natural rubber latex (NRL) was safe and efficacious in paediatric patients with NRL allergy.* ***Research Design and Methods:*** *We studied 12 NRL-allergic children (age 4-15), previously assigned to the treated arm of a double-blind placebo controlled study, who received a commercial latex SLIT for three years. Adverse reactions were monitored. The primary end-point was the NRL glove-use test. As secondary end-points, skin prick test with NRL and NRL serum specific IgE were used.* ***Main outcomes measures:*** *No SLIT-related side effects were observed. A significant reduction of the glove-use score was observed after one-year treatment (5.1 ± 4.2 vs. 14.8 ± 5.7, p=0.0031). This parameter was further reduced in the second year since SLIT start (2.0 ± 2.7, p=000007). After 3 years of SLIT all patients had a negative glove-use test (p<0.0001). Baseline wheal areas of skin prick test (6.8 ± 2.5 mm^2) were significantly reduced after 2 (5.3 ± 1.8 mm^2) and 3 years (4.0 ± 1.8 mm^2) of SLIT (p=0.039 and 0.027, respectively). Baseline values of serum specific IgE (23 ± 34 KU/l) were significantly reduced after 3 years since SLIT start (6.4 ± 5.0, p=0.0371).* ***Conclusions:*** *Three years of latex SLIT is safe and consolidates the efficacy previously observed after one year of treatment in paediatric patients.*

Introduction

Natural rubber latex (NRL) causes allergy worldwide in healthcare workers (5-17% incidence in exposed subjects) as well as outside of the healthcare environment (about 1% incidence in the general population) (1, 2), mainly in kitchen personnel (3), workers at latex manufacturing plants (4), gardeners (5), hairdressers (6) and subjects who were subjected to multiple surgeries (7).

The preventive measures to reduce latex exposure taken in the last decade by removing powered latex gloves from hospitals have significantly reduced both new sensitizations and the occurrence of severe reactions following latex exposure of sensitized subjects (8). However, the situation remained critical, since NRL is used alone or combined with other substances in the manufacturing of more than 40,000 different objects for technical, professional and everyday-life use (9).

Encouraging results have been obtained with NRL specific immunotherapy by subcutaneous (10, 11), percutaneous (12) and sublingual routes (13-16). We recently demonstrated the safety and efficacy of sublingual im-

Presentation at meetings: the present work was presented as a poster at the 2007 EAACI meeting (Gøteborg, Sweden, 9-13 June 2007), and published as a supplement in Allergy [*Allergy*, 62 (suppl. 83):167-551, abstract number 1175]

munotherapy (SLIT) with a standardized NRL extract with a double blind, placebo controlled study in a population of paediatric patients sensitized to latex who had cutaneous and, in some cases, respiratory symptoms (17).
Here, we report the results of the clinical and immunological follow-up of children who were recruited in that study and show that the efficacy of SLIT further improved and consolidated after three years of treatment, in the absence of any relevant adverse event.

Methods

Study design

This was a open, observational study on 12 patients (age 6-17 years) with clinical signs of allergy to NRL that were found eligible for sublingual immunotherapy (SLIT) with a commercial NRL extract. These patients were previously recruited for a one-year double-blind placebo controlled study (17). After the first year of treatment, the study was opened and the twelve subjects assigned to the active arm were offered to enter the present study, which lasted two more years after the end of the double blind-placebo controlled phase of the study. The parents gave their informed consent after being informed of the possible alternatives, such as allergen avoidance or symptomatic medication.

Outcomes

The outcomes of this study were the safety and efficacy of immunotherapy.
Safety was evaluated clinically by recording any adverse event that could be related to SLIT. To this aim, parents and patients were interviewed at every control visit, which was scheduled every 3 months.
Efficacy was evaluated by a quantitative structured use and rubbing test with NRL-containing gloves, based on the technique first described by Turjanmaa and co-workers (18), which was previously described in great detail (17). Briefly, patients were asked to put on one latex glove (Touchy gloves, International PBI, Milan, Italy) for 15 minutes. Then the glove was removed and the face was rubbed twice with the external part and twice with the internal part of the glove. Local (itching, erythema, wheals) and general symptoms (rhinitis, asthma) were evaluated every fifteen minutes for a period of two hours. The test was blocked by the oral administration of oxatomide and betametaxone. Each symptom scored according to previously reported values, which did not take into account the severity of each symptom but attributed to the symptom itself an absolute value incorporating the assessment of severity (17).
The following secondary outcomes were also considered:
1) Conventional skin prick tests with a NRL extract with a skin prick test solution containing a NRL extract standardized at 500 μg/ml of total protein and corresponding to 30 Histamine Equivalent Prick test Units (HEP) (ALK Abellò, Milano, Italy), prepared as previously described from ammoniated NRL (19).
The skin prick test for NRL was performed and interpreted according to the EAACI guidelines (20). Briefly, the test was performed on the volar area of the forearm by introducing the tip of a lancet with a 1-mm tip (Allergy pricker, Bayer DHS, Milan, Italy) into the skin through the allergenic or the control solution, with gentle pressure and without causing any bleeding. After thirty minutes the areas of the wheal and erythema were marked with a fine-tipped ballpoint pen and transferred onto paper with adhesive tape (Scotch Tape, 3M Italia, Italy) for subsequent planimetric determination of the wheal area. Wheals with an area of less than 7 square mm (i.e. less the 3 mm in diameter) were considered negative.
2) Specific IgE, which were measured with the Phadia Immunocap method (Phadia, Uppsala, Sweden) and expressed in kU/l

Ethics

The procedures followed were in accordance with the ethical standards of the responsible Institutional Committee on Human Experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

Treatment

SLIT-LATEX (ALK-Abellò), a commercially available NRL extract for sublingual administration was used. The extract was prepared by neutralization, semi-purification and concentration of an ammoniated NRL suspension and biologically standardized, as described elsewhere (19).
The build-up phase of the treatment was previously described as part of the first-year double-blind, placebo-controlled study (17) and was completed in 4 days according to a rush schedule. After the build-up phase patients followed the maintenance schedule, consisting of 2 drops of the maximum concentration (resulting in 40 μg of

NRL per administration) every day for a total of 36 months.
Maintenance administrations were performed at home by each patient, whose parents had been instructed on how to proceed in case of adverse effects and specifically asked to immediately report any adverse reaction or discomfort to the allergologists.

Statistical analysis

Comparisons of the results observed in different groups were then performed with Mann-Whitney two sample statistics for non-parametric data. All statistical analyses were done with the PRISM statistical software package (Graphpad Inc., San Diego, CA, USA). All statistical tests were two-sided with a significance level of 0.05.

Results

Safety of SLIT-LATEX treatment

All patients included in the active and placebo group well tolerated the treatment. There was no sign of local (buccolingual) or systemic side effects, including gastrointestinal symptoms and anaphylactic shock.

Scores of symptoms triggered by the glove use and rubbing test

A significant reduction of the symptom score of the glove use and rubbing test was observed after one-year treatment as compared to baseline (p=0.0031) (figure 1). A further reduction was measured in the second year since SLIT start (p=0.0010). After 3 years of SLIT all patients had a negative glove-use test (p<0.0001)

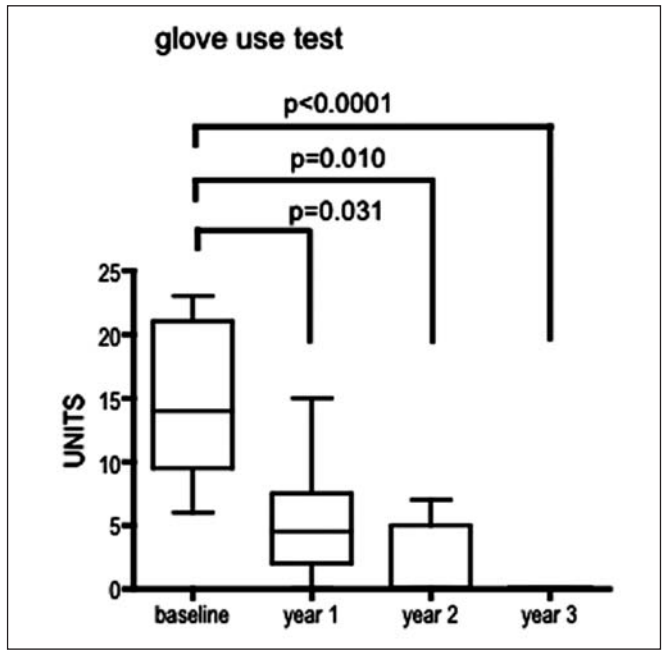
Skin reactivity to NRL

Baseline values of wheal areas measured with skin prick test with NRL extract remained unchanged after the first year of treatment (figure 2, top panel). Wheal areas were significantly reduced as compared to baseline after two and three years since treatment start (p=0.027 and 0.039, respectively).

NRL specific IgE in serum

Although a trend toward reduction was observed, values of NRL specific IgE remained unchanged as compared to baseline after the one and after two years of treatment (figure 2, bottom panel). A significant reduction of NRL specific IgE as compared to values measured at study enter was observed after three years of treatment (p=0.0371) (*ibidem*).

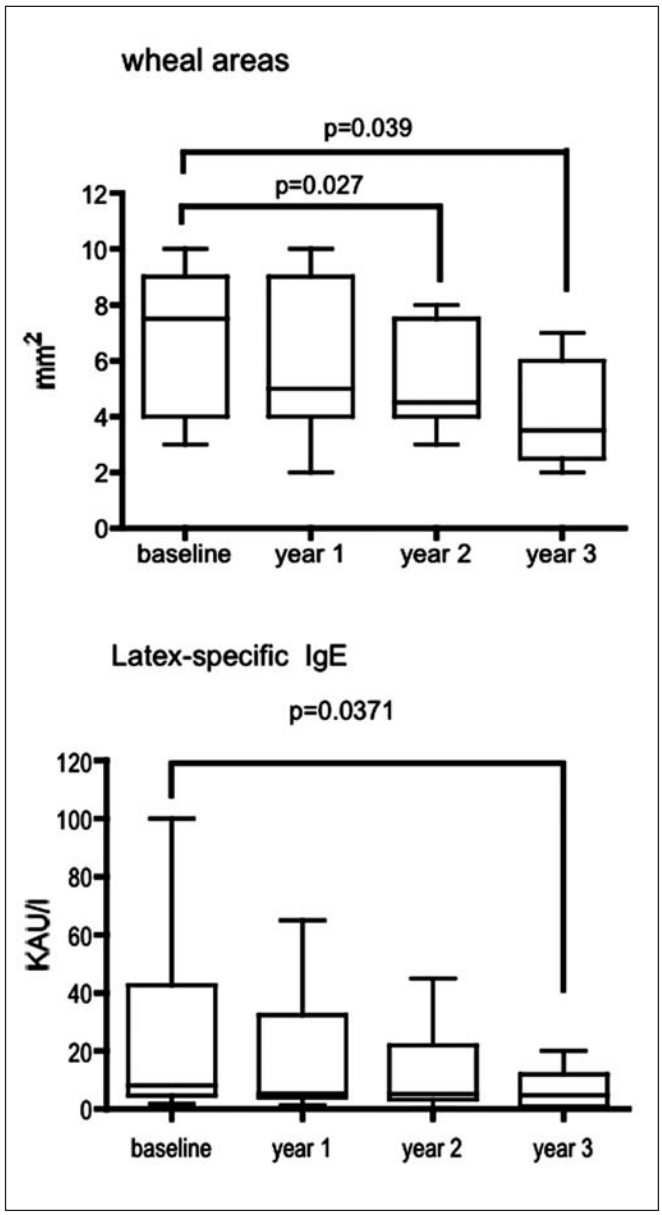
Figure 1 - Whisker-plot representation of the distribution of values of results of "Glove use test" (units on the *y*-axis) at baseline and at the indicated times of follow up (*x*-axis). Lines indicate minimum, maximum, median and interquartile ranges of the distribution of values. Results of statistical analysis for score reduction as compared to baseline are indicated



Discussion

This study is the prosecution, in the form of an open observational phase, of a previously published double-blind, placebo controlled study on specific SLIT in paediatric patients allergic to NRL (17). We found that the clinical and immunological improvement obtained after one year of NRL-specific SLIT was consolidated after three years of treatment. Moreover, no relevant adverse effects were observed. Our data confirm and strengthen the conclusions of a recent, short-term open study on paediatric patients (21).
Overall, specific immunotherapy was reported to yield successful desensitization in trials involving adult patients allergic to NRL (11, 13, 16, 22, 23). However, a relatively

Figure 2 - *Top panel.* Whisker-plot representation of the distribution of values of wheal areas (in square mm, on the y-axis) measured with NRL skin prick test at baseline and at the indicated times of follow up (x-axis). Lines indicate minimum, maximum, median and interquartile ranges of the distribution of values. P-values of comparison analysis of areas measured during follow up, as compared to baseline, are indicated.
Bottom panel. Whisker-plot representation of the levels of NRL-specific IgE (in kU/l, on the *y*-axis) at baseline at the indicated times of follow up (on the *x*-axis). Lines indicate minimum, maximum, median and interquartile ranges of the distribution of values. P-values of comparison analysis of IgE titres measured during follow up, as compared to baseline, are indicated



high frequency of systemic adverse event was observed with the subcutaneous route of administration (e.g., 46% and 8% of administered doses in ref. (11) and (24), respectively). In contrast, SLIT with NRL extracts has proven efficacious and safe in the seminal works from Patriarca's group (13, 22, 23, 25), which were recently confirmed by other investigators (16). Only one case of anaphylaxis with latex SLIT has been reported until now (26).

Our data extend these results by showing in a three-years follow-up that clinical and immunological parameters are consistently consolidated by the prosecution of NRL SLIT up to an extent of time, which is considered suitable to establish the results in SLIT protocols with other airborne allergens (27).

The rubber use test we performed mimicked real-life exposure to this allergen. Such a challenge test was necessary, since data on clinical symptoms following spontaneous NRL exposure are hardly obtained in paediatric patients, for whom allergen avoidance is more easily achieved and maintained as compared to adult individuals.

Our data clearly demonstrate that the significant reduction of symptom scores, achieved in the first year since immunotherapy start, was confirmed and extended in the following two years.

It cannot be excluded that the reduction of environmental allergen exposure could *per se* improve the reactivity to NRL in our patients. However, our data indicate that specific immunotherapy, which after one year was already capable of reducing the glove use score in treated but not in control subjects (17), was associated in the following two years to the virtual disappearance of any measurable reactivity to NRL.

Moreover, the modification of two biological parameters, which were considered in the follow up of patients included in the present study, were consistent with the clinical scores. Namely:

i) Skin reactivity to NRL was lower as compared to baseline after two (p=0.027) and three years (p=0.039) of SLIT, a result which is in agreement with observations reported in previous trials with NRL subcutaneous (24) and sublingual (16) immunotherapy;

ii) NRL specific IgE levels tended to progressively decrease in the first and second since SLIT start, and were indeed significantly reduced after 3 years of immunotherapy. Allergen specific IgE levels are not usually considered useful in the evaluation of immunotherapy in general and of SLIT in particular. Recently, *Nettis* et al. (16) reported that specific IgE did not change after SLIT with NRL (16). Similarly, NRL IgE specific levels did not change

following specific subcutaneous immunotherapy (24). However, to our knowledge this is the first time that specific IgE have been monitored in a three-year follow up of NRL SLIT.

Beneficial effects of allergen-specific immunotherapy on oral allergy symptoms have been reported (28). However, this advantage was reversible, and symptoms reappear at immunotherapy end. Also in patients included in the present study, we observed an overall trend towards improvement of food allergy to cross-reacting foods, which was already evident one year of SLIT with NRL and further increased in the second and third year (not shown). These data suggest that oral allergy can be partially improved when immunotherapy is performed with allergen components, which are immunologically cross-reactive (29).

In the NRL allergy field, it is well established that prevention from allergen exposure can induce a reduction in the incidence of sensitization. However, this environmental measure is not sufficient to warrant in single subjects re-sensitization or adverse reaction on re-exposure (30). Moreover, although the peak of the epidemic of NRL allergy was passed at least for health care workers (8), the question arises whether the history of latex allergy will repeat itself in fast developing Countries, which are increasing the use of latex products (31, 32). Thus, we believe that research on latex allergy, including accurate diagnosis (33) and specific immunotherapy should not decrease due to this partial epidemiological improvement.

Our data support the notion that NRL specific immunotherapy should enter clinical practice and no longer be utilized as an experimental therapeutic approach in paediatric patients with severe symptoms for whom allergen avoidance cannot be warranted. SLIT for NRL allergy is a safe a treatment for allergic children as it was previously reported for adults and should be extended for three years to achieve full efficacy.

Acknowledgements

ALK Abellò supported this study by providing placebo and immunotherapy extract. S.P., and M.M. are employed by ALK AbellòItaly as Medical Service Manager and Medical Director, respectively.

References

1. Charous BL. The puzzle of latex allergy: some answers, still more questions. Ann Allergy 1994; 73: 277-81.
2. Nettis E, Assennato G, Ferrannini A, Tursi A. Type I allergy to natural rubber latex and type IV allergy to rubber chemicals in health care workers with glove-related skin symptoms. Clin Exp Allergy 2002; 32: 441-7.
3. Sussman GL, Lem D, Liss G, Beezhold D. Latex allergy in housekeeping personnel. Ann Allergy Asthma Immunol 1995; 74: 415-8.
4. Tarlo SM, Wong L, Roos J, Booth N. Occupational asthma caused by latex in a surgical glove manufacturing plant. J Allergy Clin Immunol 1990; 85: 626-31.
5. Carrillo T, Blanco C, Quiralte J, Castillo R, Cuevas M, Rodriguez de Castro F. Prevalence of latex allergy among greenhouse workers. J Allergy Clin Immunol 1995; 96: 699-701.
6. Nettis E, Dambra P, Soccio AL, Ferrannini A, Tursi A. Latex hypersensitivity: relationship with positive prick test and patch test responses among hairdressers. Allergy 2003; 58: 57-61.
7. Nettis E, Dambra P, Soccio AL, Loria MP, Ferrannini A, Tursi A. Type I allergy to natural rubber latex and Type IV allergy to rubber chemicals in children with risk factors. Contact Dermatitis 2001; 44: 181-2.
8. Reunala T, Alenius H, Turjanmaa K, Palosuo T. Latex allergy and skin. Curr Opin Allergy Clin Immunol 2004; 4: 397-401.
9. Thurlow KL. Latex allergies: management and clinical responsibilities. Home Healthc Nurse 2001; 19: 369-75; quiz 76.
10. Pereira C, Rico P, Lourenco M, Lombardero M, Pinto-Mendes J, Chieira C. Specific immunotherapy for occupational latex allergy. Allergy 1999; 54: 291-3.
11. Leynadier F, Herman D, Vervloet D, Andre C. Specific immunotherapy with a standardized latex extract versus placebo in allergic healthcare workers. J Allergy Clin Immunol 2000; 106: 585-90.
12. Nucera E, Schiavino D, Pollastrini E, et al. Desensitization to latex by percutaneous route. J Investig Allergol Clin Immunol 2002; 12: 134-5.
13. Patriarca G, Nucera E, Pollastrini E, et al. Sublingual desensitization: a new approach to latex allergy problem. Anesth Analg 2002; 95: 956-60, table of contents.
14. Cistero Bahima A, Sastre J, Enrique E, et al. Tolerance and effects on skin reactivity to latex of sublingual rush immunotherapy with a latex extract. J Investig Allergol Clin Immunol 2004; 14: 17-25.
15. Savi E, Maffini I, Burastero SE. A latex-containing hepatitis-B vaccine administered in a severely latex allergic paediatric patient after specific sublingual immunotherapy: a case report. Allergy 2004; 59: 1014-5.
16. Nettis E, Colanardi MC, Soccio AL, et al. Double-blind, placebo-controlled study of sublingual immunotherapy in patients with latex-induced urticaria: a 12-month study. Br J Dermatol 2007; 156: 674-81.
17. Bernardini R, Campodonico P, Burastero S, et al. Sublingual immunotherapy with a latex extract in paediatric patients: a double-blind, placebo-controlled study. Curr Med Res Opin 2006; 22: 1515-22.
18. Turjanmaa K, Reunala T, Rasanen L. Comparison of diagnostic methods in latex surgical glove contact urticaria. Contact Dermatitis 1988; 19: 241-7.
19. Morales C, Basomba A, Carreira J, Sastre A. Anaphylaxis produced by rubber glove contact. Case reports and immunological identification of the antigens involved. Clin Exp Allergy 1989; 19: 425-30.

20. Dreborg S, Backman A, Basomba A, Bousquet J, Dieges P, Malling HI. Skin tests used in type I allergy testing. Position paper prepared by the subcommittee on Skin Tests of the EAACI. Allergy 1989; 44 (suppl 10): 1-59.
21. Nucera E, Schiavino D, Pollastrini E, et al. Sublingual desensitization in children with congenital malformations and latex allergy. Pediatr Allergy Immunol 2006; 17: 606-12.
22. Nucera E, Schiavino D, Buonomo A, et al. Latex rush desensitization. Allergy 2001; 56: 86-7.
23. Patriarca G, Nucera E, Buonomo A, et al. New insights on latex allergy diagnosis and treatment. J Investig Allergol Clin Immunol 2002; 12: 169-76.
24. Sastre J, Fernandez-Nieto M, Rico P, et al. Specific immunotherapy with a standardized latex extract in allergic workers: a double-blind, placebo-controlled study. J Allergy Clin Immunol 2003; 111: 985-94.
25. Patriarca G, Nucera E, Buonomo A, et al. Latex allergy desensitization by exposure protocol: five case reports. Anesth Analg 2002; 94: 754-8.
26. Antico A, Pagani M, Crema A. Anaphylaxis by latex sublingual immunotherapy. Allergy 2006; 61: 236-7.
27. Marogna M, Spadolini I, Massolo A, Canonica G, Passalacqua G. Long-lasting effect according to the duration of sublingual immunotherapy: A 15-year prospective study. Word Allergy Congress. Bangkok, Thailand, 2007.
28. Asero R. How long does the effect of birch pollen injection SIT on apple allergy last? Allergy 2003; 58: 435-8.
29. Lavaud F. Cross-sensitization between latex and fruits. J Allergy Clin Immunol 1996; 98: 473-4.
30. Nettis E, Colanardi MC, Ferrannini A. Type I latex allergy in health care workers with latex-induced contact urticaria syndrome: a follow-up study. Allergy 2004; 59: 718-23.
31. Chen YH, Lan JL. Latex allergy and latex-fruit syndrome among medical workers in Taiwan. J Formos Med Assoc 2002; 101: 622-6.
32. Ozkan O, Gokdogan F. The prevalence of latex allergy among health care workers in Bolu (Turkey). Dermatol Nurs 2003; 15: 543-7, 54.
33. Wagner S, Breiteneder H. Hevea brasiliensis latex allergens: current panel and clinical relevance. Int Arch Allergy Immunol 2005; 136: 90-7.

C.-H. Lee, Y. K.-C. Butt, M.-S. Wong, S. C.-L. Lo

A lipid extract of *Perna canaliculus* affects the expression of pro-inflammatory cytokines in a rat adjuvant-induced arthritis model

State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Shenzhen and Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, China

Key words
Lyprinol®, NSAID, adjuvant-induced arthritis, pro-inflammatory cytokines

Summary
As published initially in this same journal in 2000 (1), the lipid extract of Perna canaliculus (New Zealand green-lipped mussel; Lyprinol®) is known for its anti-inflammatory effects in animal models and in human controlled studies (arthritis; asthma). As a follow-up of its effects on pain in a rat model of adjuvant-induced arthritis (AIA) (2), we studied its effects on the production of cytokines known to be associated with inflammation (IL-6, IL-1α TNF-α, IFN-γ). Feeding with Lyprinol was associated with significantly decreased expression levels of TNF-α and IFN-γ when compared to Naproxen (positive control) and, even more when compared with sham and extra-virgin olive oil (negative control). When compared to Naproxen, sham and extra-virgin olive oil, the levels of IL-6 and IL-1α were also marginally decreased in rats fed with Lyprinol. This study demonstrates that AIA rats fed with Lyprinol had decreased production of cytokines associated with inflammation.

Introduction

When given to animals and humans, a lipid-rich extract prepared by supercritical fluid carbon dioxide extraction of freeze-dried stabilized New Zealand green-lipped mussel *Perna canaliculus* powder (Lyprinol®), has shown significant anti-inflammatory (AI) activity (1-7); this was described extensively for the first time in a special issue of this journal (1). According to Whitehouse and coworkers (8), when treated per os (p.o.) with this lipid extract, Wistar and Dark Agouti rats developed neither adjuvant-induced polyarthritis nor collagen (II)-induced auto-allergic arthritis. This was achieved with doses 200 times lower than other seeds or fish oils (8). In contrast to non-steroidal- anti-inflammatory drugs (NSAIDs), whole mussel extract of *Perna candaliculus* is non-gastrotoxic in disease-stressed rats at 300 mg/kg p.o. (7). Further, Lyprinol does not affect platelet aggregation in both humans and rats (5,8). Clinical studies, either controlled or randomized, have demonstrated very significant AI activity in patients with osteoarthritis (OA) (3,4,6,9), asthma (10), and other inflammatory conditions (11). There are no reported side-effects, even at doses up to 2,500 mg/day in patients. The lipid extract of *Perna canaliculus* seems to be a reproducible, stable source of bioactive lipids with much greater potency than plant/marine oils currently used as nutritional supplements to ameliorate signs of inflammation (5,8,12). This lipid extract's subfractions were also found to inhibit LTB4 biosynthesis by polynuclear white blood cells in vitro, and PGE2 production by activated macrophages (13). Much of this AI activity was found to be associated with omega-3 PUFAs and natural antioxidants, e.g. carotenoids. However, the exact mechanisms of its actions are not clear.

We conducted a series of experiments to understand the anti-inflammatory mechanism of action of this lipid extract and its effects on pain control (2). In this article, we present the effects observed on the production of IL-6, IL-1α, TNF-α, IFN-γ, the four cytokines considered to be associated with inflammation in the AIA rat model. When compared to Naproxen, sham and extra-virgin olive oil, we found that Lyprinol is effective in reducing the production of TNF-α and IFN-γ. The levels of IL-6 and IL-1α were also marginally decreased in AIA induced rats fed with Lyprinol.

Materials and Methods

Induction of inflammation in Sprague-Dawley (SD) rats

Four groups of six 6-week-old male SD rats were purchased from the Central Animal Facility (CAF) of Hong Kong Polytechnic University (HKPU). All the rats were kept and cared under conditions that fully met the requirements of the Procedures for the Care of Laboratory Animals or Animals (Control of Experiments) Regulations Chapter 340 of the Hong Kong SAR government. Ethics approval (ASESC No.04/9) had been obtained from The Animal Subjects Ethics subcommittee of the HKPU. Arthritis was induced in anesthetized animals by administration of adjuvant according to the method previously described (8) with minor modifications. Briefly, at day 0, each rat was injected in the paw of the right hind limb with 100 µl of Freund's complete adjuvant (Sigma, St. Louis, MO, USA) containing 10 mg/ml of Mycobacterium butyricum (Difco, Livonia, MI, USA). Another six rats without arthritis induction were observed as normal group.

Products/drug tested fed to the treatment and control groups of rats

Rats in the lipid extract of *Perna canaliculus* (Lyprinol®, Pharmalink International Ltd., Burleigh Heads, QLD, Australia) study group were fed by oral gavage at a dosage of 25 mg Lyprinol / kg body weight. Typically, the required amount of Lyprinol was made up with olive oil (Virgin®, Bertolli, Italy) to 300 µl before being force-fed to the rats with a stainless steel stomach tube. 300 µl of olive oil, and 20 mg/ kg body weight of Naproxen were fed as vehicle and positive control respectively. Naproxen is a NSAID that is routinely being used to treat inflammation and known to have gastro-toxic side effects. Normal chow was provided to all the animals.

Splenocyte preparation

At day 7 and 14 after arthritis induction, splenocytes of each rat in the Lyprinol®, Naproxen and olive oil were prepared as described previously (2,14). Briefly, spleens were aseptically cut off and minced into tiny pieces. Single cell suspension was prepared by gentle grinding of spleen pieces in RPMI 1640 medium (Life Technologies, Invitrogen, Carlsbad, CA, USA). Splenocytes (mostly B and T cells) from the crude spleen cell suspension were recovered by using Ficoll-Hypaque® Plus lymphocyte isolation kit (Pharmacia Biotech, Piscataway, NJ, USA) as described in the manufacturer's manual. Recovered splenocytes (1.5 ml) were transfered into a sterile centrifuge tube and 4 volumes of pre-cold 0.83% ammonium chloride (NH_4Cl) were added and incubated for 10 minutes for the lysis of residual erythrocytes (2). Splenocytes were recovered by centrifugation at 1000 x g for 5 minutes, washed with 8 ml pre-warm (37°C) sterile PBS buffer (137mM NaCl, 2.7mM KCl, 4.3mM Na_2HPO_4–$7H_2O$, 1.4mM KH_2PO_4, pH 7.4) and finally re-suspended in appropriate volume of complete RPMI 1640 medium (10% fetal bovine serum, 100units/ml of penicillin and 100µg/ml of streptomycin, supplemented with L-glutamine and 25 mM HEPES).

Cell count and viability staining

Re-suspended splenocytes were stained with 0.4% Trypan blue exclusion dye (0.4 g Trypan blue in 100ml PBS buffer) at ratio 1:1. The number of splenocytes was counted and calculated with the aid of a hemocytometer. More than 99% of splenocytes were viable. Except stated otherwise, splenocytes were diluted to a working population of 5 x 106 cells/ ml in the following experiments.

ELISA assay for cytokines

5 x 106 splenocytes/ ml were seeded in 24-well plate; a suboptimal concentration of 1.25 µg/ ml of lipopolysaccharide (LPS) (Sigma, St. Louis, MO, USA) was used to prime the splenocyte culture. Cell culture was incubated in 37? incubator at 80% humidity and 5% CO_2 atmosphere

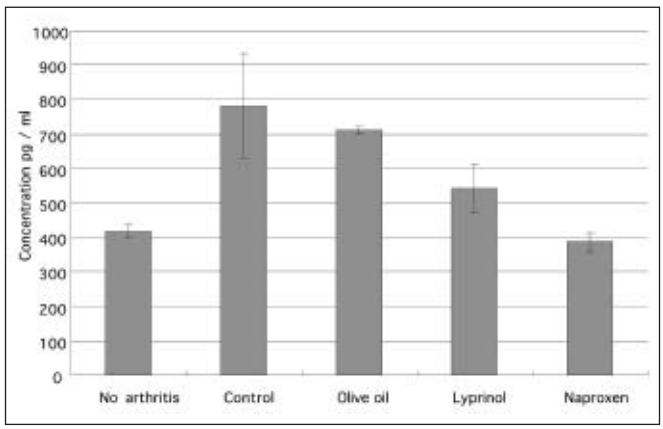
condition. After incubating with LPS, supernatants were collected either at 10 hours or at a specific time wherever indicated in the text, before being stored at -80 °C until use. IL-6, IL-1α, TNF-α and IFN-γ were measured by an enzyme-linked immunosorbent assay (ELISA) sandwich type assay (BioSource, Camarillo, CA, USA) as described in the user's manual. Samples used for measurements of IL-6, TNF-α and IFN-γ were supernatants of splenocytes that had been incubated with LPS for 10 hours. On the other hand, because of the low levels of expression of IL-1α, samples used for measurements of IL-1α were supernatants of splenocytes that had been incubated with LPS for 24 hours. Data obtained were compared to those of the control group and analyzed by Student's t-test.

Results

Levels of pro-inflammatory cytokine interleukin-6 (IL-6)

Figure 1 shows that the level of the pro-inflammatory cytokine IL-6 was decreased at day 7. The level of IL-6 in the Lyprinol® group was significantly lower than the one of the control and olive oil groups. The level of IL-6 in the Lyprinol® group is close to the one observed in the NSAID Naproxen group. Our results clearly demonstrated that Lyprinol® can decrease the production of the pro-inflammatory cytokine IL-6 in the early phase of AIA.

Figure 1 - Amount of pro-inflammatory cytokine interleukin-6 (IL-6) produced by LPS-stimulated splenocytes after 7 days of arthritis induction. Although not statistically significant, Lyprinol lowered the amount of IL-6 production when compared to control and olive oil groups, though not as effectively as the NSAID Naproxen. Data shown are mean + S.E.M. (n=6)



Levels of pro-inflammatory cytokine interleukin-1α (IL-1α)

IL-1α level of AIA rat splenocytes at days 7 and 14 was measured. Figure 2 shows that the level of IL-1α at day 7 and especially at day 14 was reduced significantly in the Lyprinol® group when compared to the control and olive oil groups. This effect on IL-1α production was not seen in rats fed with the NSAID Naproxen. The results demonstrated that Lyprinol can reduce the production of the pro-inflammatory cytokine IL-1α in AIA rats.

Levels of cytokines tumor necrosis factor-α (TNF-α) and interferon-gamma (IFN-γ)

As shown in figure 3, levels of TNF-α in the Lyprinol® group on day 14 were greatly decreased when compared to those of the control group. Indeed, the level of TNF-α de-

Figure 2 - Amount of pro-inflammatory cytokine IL-1-alpha produced by LPS-stimulated splenocytes at (A) day 7 and (B) Day 14 after induction of arthritis in rats

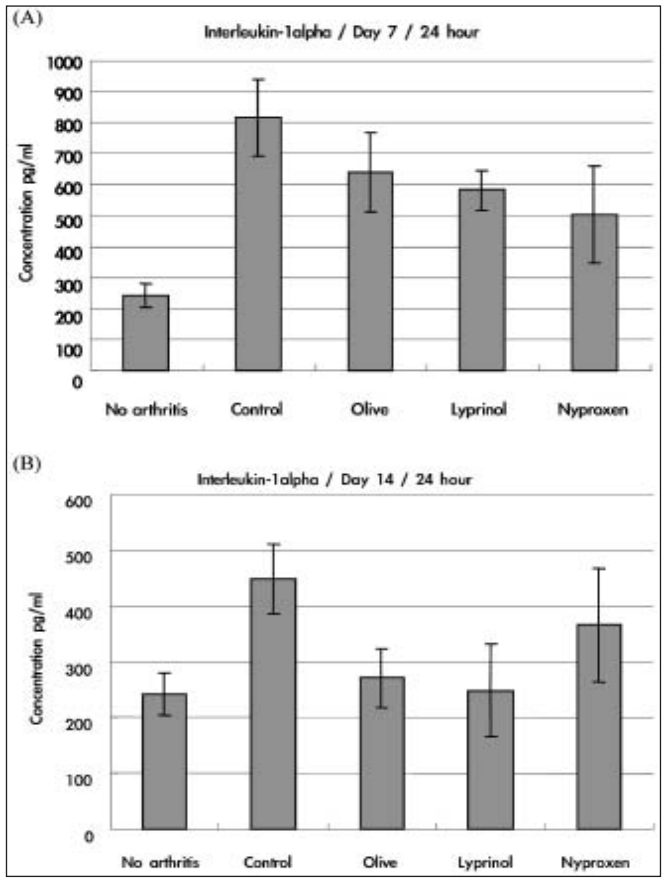


Figure 3 - Amount of pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) produced by post 10 hours LPS-stimulated splenocytes collected after 14 days of arthritis induction. Lyprinol effectively controlled the amount of TNF-α produced when compared to control and olive oil groups. More importantly, Lyprinol was more effective than the NSAID Naproxen. Data are mean + S.E.M. (n=6). * $p < 0.05$

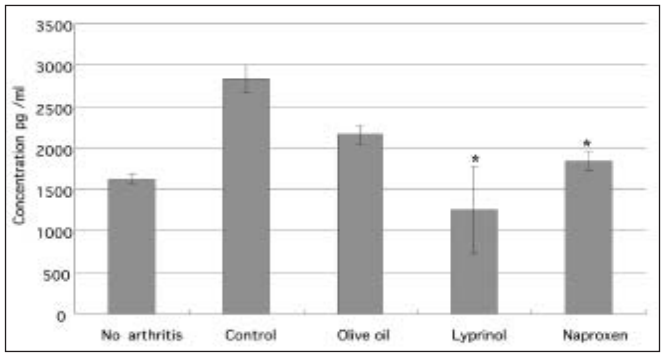
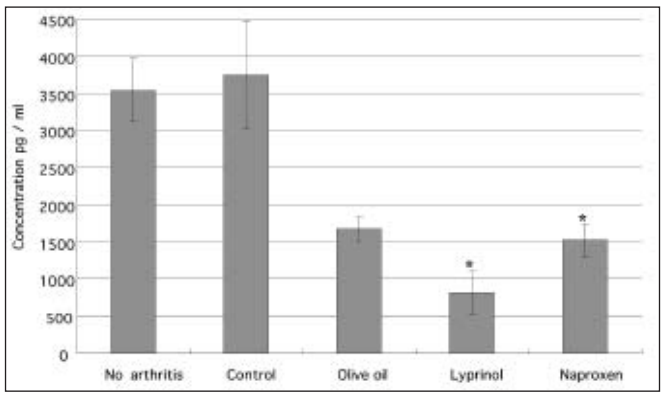


Figure 4 - Amount of interferon-gamma (IFN-γ) produced by post 10 hours LPS-stimulated splenocytes collected after 14 days of arthritis induction. As can be seen, Lyprinol effectively lowered the amount of IFN-γ produced when compared to control and olive oil groups. Strikingly, Lyprinol controlled the IFN-γ production even more effectively than the NSAID Naproxen. Values shown are mean + S.E.M. (n=6). * $p < 0.05$



tected was lower than the one found in AIA rats treated with the NSAID Naproxen. Besides, the production of another pro-inflammatory cytokine, IFN-γ, was also found to be significantly decreased. As shown in figure 4, IFN-γ level of Lyprinol® group was dramatically decreased when compared to the control and olive oil-fed groups. Again, the level of INF-γ in the Lyprinol® group was even lower than the one observed in the AIA rats treated with the NSAID Naproxen. Our results showed that Lyprinol® can decrease the production of IL-6, TNF-α, IL-1α and IFN-γ in AIA rats.

Discussion

AIA is a loco-regional highly inflammatory experimental condition, with systemic repercussions. AIA in the rats is a standard model accepted for the study of inflammation, and its control by medications or supplements (2,8,15). The lipid extract of *Perna canaliculus* (the green-lipped mussel of New Zealand; Lyprinol®) is known as a powerful anti-inflammatory product in animal models and human diseases (asthma, arthritis). We used the AIA rat model in order to get a better understanding of the mechanisms resulting in the anti-inflammatory effects of Lyprinol®. We measured the levels of cytokines that are known to be pro-inflammatory: IL-1α, IL-6, TNF-α and IFN-γ. We compared results observed when using Lyprinol with the ones observed with sham, olive oil (negative control), and the non-steroid anti-inflammatory drug (NSAID) Naproxen (positive control); and we observed a group of rats that had no AIA as reference. We found that the group of rats given Lyprinol had a significantly decreased production of some of pro-inflammatory cytokines; this provides a partial explanation on how Lyprinol can help to control the symptoms related to inflammation (2, 4-7).

Lyprinol® did control the production of pro-inflammatory cytokines better than Naproxen in AIA rats. Extra-virgin olive oil was ineffective.

The AIA rat model has been extensively studied, both to assess the efficacy of medications and monitor inflammation-associated cytokines. For example, Anderson (16) found that SC-58125, a selective COX-2 inhibitor, inhibited IL-6 and IL-6 mRNA. Avramidis (17) found that grape melanin normalized elevated levels of IL-6 and TNF-α. Badger (18) found that idoxifene, a selective estrogen receptor modulator, reduced serum IL-6 levels in animals treated with 10 mg idoxifene/kg body weight/day. D-43787, a cyclosporine receptor-binding immunomodulator was found to inhibit LPS-induced IL-6 and TNF-α production (19). Bindarit, an inhibitor of MCP-1, was found to decrease TNF-α production after LPS induction (20). Kim et al. (21) described the antinociceptive and anti-inflammatory effects of ethylacetate extracts from Bang-Poon (*Radix lebouriellae*) on IL-6. Prophylactic and 6-day therapeutic treatment with FK506 (tacrolimus) was found to reduce the levels of IL-6 and TNF-α (22). An extract from an Indian plant, *Swertia chirayita*, was also found to reduce in a dose-dependent fashion, the levels of TNF-α, IFN-γ and IL-1, while IL-6 was only affected when higher doses (23.72 and 35.58 mg/kg) were administered (23). Magari and coworkers found that leflunomide inhibited anti-

CD3/CD28 induced production of TNF-α, IL-6 and IL-1, (24). Barsante et al. found that atorvastatin significantly decreased the concentrations of IL-6, TNF-α and IL-1, (25). The Chinese herbal preparation QFGJS was also reported to decrease significantly the serum levels of IL-6, TNF-α, IL-1α (26). A targeted DNA vaccine using naked DNA which encodes for TNF-α resulted in the generation of immunological memory to its gene product which effectively inhibits the development of AIA (27). Other studies used different assays to evaluate the control of production of these pro-inflammatory cytokines: for instance a novel inhibitor of p38 MAP kinase, TAK-715 (28) inhibited LPS-stimulated release of TNF-α from human monocytic THP-1 cells in vitro. The benefits observed in animal models associated with the reduction in the production of TNF-α resulted in the suggestion (29) of a beneficial association between pentoxiphylline and Lyprinol®, instead of low-dose prednisolone. All these previous studies on the same AIA animal model have been using substances that lack the impressive clinical baggage that Lyprinol carries, both in terms of efficacy, and safety.

Nevertheless, it should be stressed that AIA rats receiving Naproxen experienced multiple hemorrhagic ulcerations of the gastro-intestinal tract on post-mortem examinations (after harvest of the splenocytes) while the ones receiving Lyprinol® fared very well. Further, in our previous studies, rats taking Lyprinol recovered from AIA after one year of administration (14).

Conclusion

Administration of Lyprinol, the lipid complex of *Perna canaliculus*, to rats with adjuvant-induced arthritis resulted in a diminution of production of some cytokines (i.e. IL-6, IL-1α, TNF-α, and IFN-γ) known to be associated with inflammation. This effect is more pronounced than the one Fed with Naproxen (positive control), and much stronger than the one Fed with extra-virgin olive oil (other control). Our results suggest that further investigations on Lyprinol as a treatment of arthritis should be considered.

Acknowledgements

Georges M. Halpern, MD, PhD, initiated this study, organized it, and wrote the manuscript; he should have been the leading author. We thank Pharmalink International Ltd. for supplying the lipid extract of *Perna canaliculus* and the placebo, and an unrestricted educational and research grant that supported our study in part. We would also like to express our sincere thanks to Dr. John Honkei Lum for this contribution to this work when he was part of our team.

References

1. Allergie & Immunologie (Paris), 2000; 32: 259-294.
2. Lee CH, Lum JHK, Ng CKC, McKay J, Butt YKC, Wong MS, Lo SCL. Pain controlling and cytokine-regulating effects of Lyprinol, a lipid extract of *Perna canaliculus*, in a rat adjuvant-induced arthritis model. eCAM 2007;doi:10.1093/ ecam/nem100 Advance access published September 26, 2007.
3. Cho SH, Jung YB, Seong SC, Park HB, Byun KY, Lee DC, Song EK, Son JH. Clinical efficacy and safety of Lyprinol, a patented extract from New Zealand green-lipped mussel (*Perna canaliculus*) in patients with osteoarthritis of the hip and knee: A multicenter 2-month clinical trial. Allergie & Immunologie (Paris) 2003;35:212–6.
4. Gibson SLM, Gibson RG. The treatment of arthritis with a lipid extract of *Perna canaliculus*: a randomized trial. Complementary Therapies in Medicine 1998;6: 122.
5. Halpern GM. Anti-inflammatory effects of a stabilized lipid extract of *Perna canaliculus* (Lyprinol®). Allergie & Immunologie (Paris) 2000;32:272–8.
6. Lau CS, Chiu PKY, Chu EMY, Cheng IYW, Tang WM, Man RYK, Halpern GM. Treatment of knee osteoarthritis with Lyprinol®, lipid extract of the green-lipped mussel -a double-blind placebo-controlled study. Progress in Nutrition 2004;6: 17–31.
7. Rainsford KD, Whitehouse MW. Gastroprotective and anti-inflammatory properties of green-lipped mussel (*Perna canaliculus*) preparation. Arzneimittelforschung 1980;30:2128–32.
8. Whitehouse MW, Macrides TA, Kalafatis N. Anti-inflammatory activity of a lipid fraction (Lyprinol®) from the N. Z. green-lipped mussel. Inflammopharmacology 1997;5:237–246.
9. Gruenwald J, Graubaum HJ, Hansen K, Grube B. Efficacy and tolerability of a combination of Lyprinol and high concentrations of EPA and DHA in inflammatory rheumatoid disorders. Advances in Therapy 2004;21:197–201.
10. Emelyanov A, Fedoseev G, Krasnoschekova O, Abulimity A, Trendeleva T, Barnes PJ. Treatment of asthma with lipid extract of New Zealand green-lipped mussel: a randomised clinical trial. European Respiratory Journal 2002;20: 596–600.
11. Halpern GM. The Inflammation Revolution. Square One Publishers, Garden City, NY., USA. 2005.
12. Sinclair AJ, Murphy KJ, Li D. Marine lipids: overview: new insights, and lipid composition of Lyprinol®. Allergie & Immunologie (Paris) 2000;32:261–271.
13. Dugas B. Lyprinol inhibits LTB4 production by human monocytes. Allergie & Immunologie 2000;32:284–9.
14. Zimecki M, Weber-Dabrowska B, Lusiak-Szelachowska M, Mulczyk M, Boratynski J, Pozniak G, Syper D, Gorski A. Bacteriophages provide regulatory signals in mitogen-induced murine splenocyte proliferation. Cellular & Molecular Biology Letters

2003;8:699-711.
15. Whitehouse MW. Adjuvant-induced polyarthritis in rats. In: Greenwald RA, Diamond HS (Eds.) CRC Handbook of Animal Models for the Rheumatic Diseases Vol. 1, CRC Press, Miami, FL. 1996.
16. Anderson GD, Hauser SD, McGarity KL, Bremer ME, Isakson PC, Gregory SA. Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin-6 in rat adjuvant arthritis. Journal of Clinical Investigation 1996;97:3672-9.
17. Avramidis N, Kourounakis A, Hadjipetrou L, Senchuk V. Anti-inflammatory and immunomodulating properties of grape melanin. Inhibitory effects on paw edema and adjuvant-induced disease. Arzneimittelforschung 1998;48:764-71.
18. Badger AM, Blake SM, Dodds RA, Griswold DE, Swift BA, Rieman DJ, Stroup GB, Hoffman SJ, Gowen M. Idoxifene, a novel selective estrogen receptor modulator, is effective in a rat model of adjuvant-induced arthritis. Journal of Pharmacology and Experimental Therapeutics 1999;291:1380-6.
19. Pahl A, Zhang M, Torok K, Kuss H, Friedrich U, Magyar Z, Szekely J, Horvath K, Brune K, Szlenyi I. Anti-inflammatory effects of a cyclosporine receptor-binding compound, D-43787. Journal of Pharmacology and Experimental Therapeutics 2002;301:738-46.
20. Guglielmotti A, D'Onofrio E, Coletta I, Aquilini L, Milanese C, Pinza M. Amelioration of rat adjuvant arthritis by therapeutic treatment with bindarit, an inhibitor of MCP-1 and TNF-alpha production. Inflammation Research 2002;51:252-8.
21. Kim HW, Kwon YB, Ham TW, Roh DH, Yoon SY, Han HJ, Kang SK, Lee HJ, Mar WC, Yang IS, Beitz AJ, Lee JH. The antinociceptive and anti-inflammatory effect of ethylacetate extracts from Bang-Poong (Radix lebouriellae) on the Freund's adjuvant-induced arthritis in rats. Journal of Veterinary Science 2002;3:343-9.
22. Magari K, Miyata S, Nishigaki F, Ohkubo Y, Mutoh S, Goto T. Differential effects of FK506 and methotrexate on inflammatory cytokine levels in rat adjuvant-induced arthritis. Journal of Rheumatology 2003;30:2193-200.
23. Kumar IV, Paul BN, Asthana R, Saxena A, Mehrotra S, Rajan G. Swertia chrayita mediated modulation of interleukin-1beta, interleukin-6, interleukin-10, interferon-gamma, and tumor necrosis factor-alpha in arthritic mice. Immunopharmacology & Immunotoxicology 2003;25:573-83.
24. Magari K, Miyata S, Nishigaki F, Ohkubo Y, Mutoh S. Comparison of anti-arthritic properties of leflunomide with methotrexate and FK506: effect on T cell activation-induced inflammatory cytokine production in vitro and rat adjuvant-induced arthritis. Inflammation Research 2004;53:544-50.
25. Barsante MM, Roffe E, Yokoro CM, Tafuri WL, Souza DG, Pinho V, Castro MS, Teixeira MM. Anti-inflammatory and analgesic effects of atorvastatin in a rat model of adjuvant-induced arthritis. European Journal of Pharmacology 2005;516:282-6.
26. Cai X, Zhou H, Wong YF, Xie Y, Liu ZQ, Jiang ZH, Bian ZX, Xu HX, Liu L. Suppressive effects of QFGJS, a preparation from an anti-arthritic herbal formula, on rat experimental adjuvant-induced arthritis. Biochemical and Biophysical Research Communications 2005;337:586-94.
27. Wildbaum G, Youssef S, Karin N. A targeted DNA vaccine augments the natural immune response to TNF-alpha and suppresses ongoing adjuvant arthritis. Journal of Immunology 2000;165:5860-6.
28. Miwatashi S, Arikawa Y, Kotani E, Miyamoto M, Naruo K, Kimura H, Tanaka T, Asahi S, Ohkawa S. Novel inhibitor of p38 MAP kinase as an anti-TNF-alpha drug: discovery of N-[4-[2-ethyl-(3-methylphenyl)-1,3- thiazol-5yl]-2- pyridyl]benzamide (TAK-715) as a potent and orally active anti-rheumatoid arthritis agent. Journal of Medicinal Chemistry 2005;48:5966-79.
29. Whitehouse MW. Anti-TNF-alpha therapy for chronic inflammation: reconsidering pentoxiphylline as an alternative to therapeutic protein drugs. Inflammopharmacology 2004;12:223-7.

Press release: Oralair® Grasses 300IR highly positive clinical results in a pharmacodynamic study conducted in an allergen challenge chamber

Antony, France; August 27th, 2008 – Stallergenes S.A. announces the highly positive results of a new study conducted in an Allergen Challenge Chamber (ACC).
The study on Oralair® Grasses included 86 adult patients exposed to grass pollen challenge with different treatment durations: 1 week, 1 month, 2 and 4 months without a titration phase. Use of rescue medication was not allowed.
The outcomes of this trial in line with the objectives were:
- An onset of action starting on the seventh day (statistically significant at one month)
- An efficacy plateau reached on challenge at the first month
- A highly statistically significant effect of Oralair® Grasses versus placebo (p=0.0003) on the average symptom score (RTSS). The magnitude of efficacy (34%)1 was similar to the findings of the previous studies VO34 (in adults) and VO52 (in a paediatric population)
- A Cohen score of 0.79 corresponding to a large effect size

Safety results, with only well-known local adverse events, and efficacy results were consistent with the findings of Oralair® Grasses studies previously conducted in adults (VO34) and children (VO52) in phase III trials.
"This study has demonstrated a clear consistency with the outcomes of previous pivotal studies. It opens the way for ACC as a new tool for phase II studies reducing the unpredictability of pollination. Moreover, it confirms the therapeutic effect of Oralair® Grasses and the very fast onset of action on the symptoms," said Olivier de Beaumont. 1 on median scores

About the study

This was a randomised, double-blind, parallel-group, placebo-controlled, single centre, phase I study to assess, subsequent to allergen challenge in an allergen exposition chamber, the efficacy and time course of sublingual immunotherapy (SLIT) administered as 300IR allergen-based tablets once daily to 86 adults suffering from grass pollen rhinoconjunctivitis. The main objectives of the study were to assess the efficacy of grass pollen extract SLIT tablets compared to placebo on the average Rhinoconjunctivitis Total Symptom Score (sneezing, rhinorrhea, nasal pruritus, nasal congestion, ocular pruritus and watery eyes), to evaluate the onset of action after different treatment durations (1 week, 1 month, 2 and 4 months) through the challenge test, to identify immunological parameters as possible biomarkers for SLIT, and to assess safety. Since patients were not allowed to take any rescue medication, the symptom score was not impacted by its usage and the difference between the placebo and active group was due exclusively to the effect of the Oralair® Grasses tablet.

About the Allergen Challenge Chamber (ACC)

The Allergen Challenge Chamber is already recognized as a supportive study by the FDA and the European Medicines Agency (EMEA). The interest of ACC as an assessment tool for clinical development is confirmed. The Allergen Challenge Chamber (ACC) offsets the unpredictability of the pollen season, and thereby avoids the variable nature of pollens and patient exposure and facilitates follow up.

About Oralair® Grasses

Oralair® Grasses is a fast-dissolving tablet that has demonstrated high efficacy in treating allergic rhinoconjunctivitis to grass pollen starting with the first season, lasting throughout the pollen season, and at the pollen peak, on:
- Poly- and mono-sensitised patients, as well as asthmatic patients,
- Every individual symptom, and in particular on nasal congestion and watery eyes.

Oralair® Grasses is a pre-seasonal treatment: it has to be started four months before the pollen season, be maintained throughout the season, and then stopped and restarted the following season.
Oralair® Grasses contains a mix of 5 standardised grass allergens: perennial rye grass (*Lolium perenne*), meadow grass (*Poa pratensis*), timothy grass (*Phleum pratense*), cocksfoot (*Dactylis glomerata*) and sweet vernal grass (*Anthoxanthum odoratum*), as a daily dose of 300 IR, so as to mimic patients' natural exposure.
In June 2008, Stallergenes was granted a marketing authorisation for Oralair® Grasses in adults, by PEI (Paul Ehrlich Institute), the biological branch of the German health agency.
Based on the positive results of the paediatric pivotal study (VO52), Stallergenes has applied in July 2008 for the paediatric extension of the product's indications.
The clinical development programme has already enrolled around 1600 patients to date. A long term pivotal study is proceeding according to schedule, and is currently in its second year. The company plans to file two INDs for adult and paediatric trials with the FDA this year.

About Stallergenes

Stallergenes is a European biopharmaceutical company dedicated to desensitisation therapies for the prevention and treatment of allergy-related respiratory diseases, e.g. rhinoconjunctivitis and allergic asthma. A pioneer and leader in sublingual desensitisation treatments, Stallergenes devotes 16% of its turnover to Research and Development and is actively involved in the development of a new therapeutic class: sublingual desensitisation tablets.
In 2007, Stallergenes had a turnover of 147 million euros and provided desensitisation treatments to more than 500,000 patients.
Stallergenes is listed on Euronext Paris (Compartment B) and is part of the sample composing the SBF 120 index.

ISIN Code: FR0000065674
Reuters Code: GEN.PA
Bloomberg Code: GEN.FP

Additional information is available at http://www.stallergenes.com

Contacts
Albert Saporta - Chairman and C.E.O Tel: +33 1 55 59 20 04

Christian Thiry - Chief Financial Officer Tel: +33 1 55 59 20 95
Email: investorrelations@stallergenes.fr

Stallergenes Press Relations
Lise Lemonnier - Communications Manager
Tel.: + 33 1 55 59 20 96 –
Email: llemonnier@stallergenes.fr

Investors and analysts relations
Lucile de Fraguier - Pavie Finance Tel.: + 33 1 42 15 04 39 –
Email: contact@pavie-finance.com

News

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, October 2, 2008

AAAAI: Look out for latex in unexpected places

MILWAUKEE – Allergic reactions to latex happen commonly in medical settings, where rubber gloves are in abundant supply. But less-visible elements in other environments can also pose danger, according to the American Academy of Allergy, Asthma & Immunology (AAAAI).

"Consider that restaurant meals are frequently prepared by cooks wearing latex gloves. In schools, the cafeteria may be a threat, but there is also potential exposure to latex in school supplies," said Donald H. Beezhold, PhD, FAAAAI, chair of the AAAAI Latex Allergy Committee. "This type of inadvertent exposure poses a serious health risk to millions of Americans."

Estimates of latex allergy prevalence vary, but the condition disproportionately affects healthcare workers and others with frequent exposure to latex – including those who have had multiple surgeries. At least 10 percent of healthcare workers and more than half of individuals with spina bifida are believed to have the allergy, versus 1 percent to 6 percent of the general population.

Reactions to latex can result in skin irritation or anaphylaxis – which can be life-threatening.

Avoidance is key to preventing an allergic reaction and the responsibility of education often falls to the patient. The AAAAI offers resources on latex allergy in the Diseases 101 section of its Web site, www.aaaai.org. The AAAAI recommends these tips for latex-allergic patients:

- Avoid contact with latex products, including gloves, balloons and condoms.
- Inform your doctors, dentist, family, employer and school personnel of the allergy and request accommodations as needed.
- Remember that the federal Americans with Disabilities Act provides workplace protections for individuals with latex allergy. If protective gloves are required for your job, your employer should consider an alternative synthetic material, which is equally effective in most situations.
- Ask your physician if you should wear a medical bracelet identifying your allergy.

An allergist/immunologist is the best-qualified medical professional to diagnose and treat latex allergy and other allergic diseases. To locate an allergist/immunologist in your area, visit the AAAAI Physician Referral Directory.

The American Academy of Allergy, Asthma & Immunology represents allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Established in 1943, the AAAAI has nearly 6,500 members in the United States, Canada and 60 other countries.

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, October 30, 2008

New research: Early peanut consumption may prevent allergy

MILWAUKEE – New research casts doubt on government health recommendations that infants and new mothers avoid eating peanuts to prevent development of food allergy.

The study, published in the November issue of The Journal of Allergy and Clinical Immunology, shows that children who avoided peanut in infancy and early childhood were 10 times as likely to develop peanut allergy as those who were exposed to peanut.

Researchers measured the incidence of peanut allergy in 8,600 Jewish school-age children in the United Kingdom and Israel. They compared these results with data on peanut consumption collected from mothers of infants age 4 to 24 months.

Prevalence of peanut allergy in the United Kingdom was estimated at 1.85 percent, versus .17 percent in Israel.

"The most obvious difference in the diet of infants in both populations occurs in the introduction of peanut," lead author George Du Toit, MD, FAAAAI, wrote in the article. At 9 months of age, 69 percent of Israeli children were eating peanut, compared to 10 percent of those in the U.K.

Dietary guidelines in the United Kingdom, Australia and – until earlier this year – the United States advise avoidance of peanut consumption during pregnancy, breastfeeding and infancy. While researchers suggest these recommendations could be behind the increase in peanut allergy in these countries, they cautioned that further evidence is needed before those guidelines should be changed.

The American Academy of Allergy, Asthma & Immunology (AAAAI) cautions that although the results are promising, they shouldn't translate to changes in treatment just yet. There are a number of other factors that could account for the difference in peanut allergy prevalence between the two countries.

"While this study's findings provide optimism for prevention of peanut allergy in the future, randomized, controlled trials are needed to verify that early introduction of peanut is indeed effective," said Jacqueline A. Pongracic, MD, FAAAAI, vice chair of the AAAAI Adverse Reactions to Foods Committee.

The Learning Early about Peanut Allergy (LEAP) study, a large randomized study in the U.K., is currently testing the effects of early peanut exposure.

Researchers selected the two Jewish populations due to their similar genetics, rate of atopy, and environmental and socioeconomic backgrounds. These similarities help eliminate other factors that could account for the difference in peanut allergy rates.

Peanut allergy affects an estimated 3 million Americans, according to the AAAAI. It is one of the most common triggers of anaphylaxis, a potentially life-threatening reaction. The incidence of peanut allergy has been on the rise in the United States, doubling in the five-year period from 1997-2002.

An allergist/immunologist is the best-qualified medical professional to diagnose and treat food allergies and other allergic diseases. To locate an allergist/immunologist, visit the AAAAI Physician Referral Directory at www.aaaai.org/physref.

The Journal of Allergy and Clinical Immunology is the official scientific journal of the American Academy of Allergy, Asthma & Immunology (AAAAI).

The AAAAI represents allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Established in 1943, the AAAAI is the largest professional medical association in the asthma/immunology specialty with nearly 6,500 members in the United States, Canada and 60 other countries.

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, November 12, 2008

AAAAI: 'Allergy-free' dog an unlikely find for First Family

MILWAUKEE – As President-Elect Barack Obama and the future First Family begin their search for a new pet to join them at the White House, the American Academy of Allergy, Asthma & Immunology (AAAAI) wishes to remind individuals with allergies that there is no truly "hypoallergenic" dog.

Obama's 10-year-old daughter, Malia, suffers from allergies to dogs – a condition she shares with millions of Americans.

According to the AAAAI, it is a common misconception that people are allergic to a dog's hair, and it is falsely believed that a dog that sheds less will not cause a reaction. However, allergies to pets are caused by protein found in the animal's dander (dead skin cells), saliva or urine.

These proteins are carried on microscopic particles through the air. When inhaled, they trigger reactions in allergic people. As all dogs posses these proteins, there is no allergy-free dog.

Though some dog breeds are considered more allergy friendly, it is likely because they are groomed more frequently – a process that removes much of the dander.

While the most effective treatment for animal allergies is avoidance, this is not always possible. The AAAAI offers these other tips for minimizing allergy symptoms:

- Visit an allergist/immunologist to diagnose the allergy and discuss treatment, which may include maintenance medications or immunotherapy (allergy shots).
- Keep the pet out of the allergic person's bedroom. Animal dander will collect on pillows, leading to worsened symptoms at night and morning.
- Bathe the animal weekly to reduce the amount of dander shed at home.
- Replace carpeting with hardwood or other solid-surface flooring for easy clean-up.
- Vacuuming may not be effective in decreasing allergen levels, but using a HEPA filter and double bags may help.
- Wash bedding and clothing in hot water. While animal allergens are not easily removed by high temperatures, these measures may help.

An allergist/immunologist is the best-qualified medical professional to diagnose and treat animal allergies. Additional information on animal allergies is available at www.aaaai.org.

The AAAAI represents allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Established in 1943, the AAAAI is the largest medical association dedicated to the allergy/immunology specialty, with nearly 6,500 members in the United States, Canada and 60 other countries.

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, November 12, 2008

Guess who's coming to dinner: Hosting guests with allergies and asthma

MILWAUKEE – This holiday season, many Americans will host gatherings with family and friends. Millions will have guests with allergy or asthma concerns.

One in six Americans – about 50 million people – suffer from some form of allergies or asthma, according to the American Academy of Allergy, Asthma & Immunology (AAAAI). But for

those who do not cope with the conditions daily and first-hand, it can be difficult to know what special accommodations are needed.
What if a dinner guest has a food allergy? Or your mother-in-law is allergic to your cat? The AAAAI offers these tips for holiday hosts to keep their guests' asthma and allergy symptoms in hibernation:

- Ask about food allergies before planning a menu. Remember that even trace amounts of an offending food can trigger an allergic reaction. Keep track of ingredients used and avoid cross-contamination by thoroughly washing utensils, cookware and food storage between uses.
- Dust and vacuum regularly in the weeks leading up to the gathering. This will help minimize dust mites, animal dander and other potential allergy triggers. Don't forget to vacuum upholstered furniture and drapery.
- Replace furnace air filters so they can properly trap allergens.
- Clean guest rooms thoroughly the day before visitors arrive, including dusting, vacuuming and washing sheets and pillowcases in hot water.
- If visitors are sensitive to animals, keep all pets out of guests' rooms. Do not allow cats or dogs to rub against guests or climb in their laps. If necessary, keep pets confined to another area of the house, such as the basement.
- Limit fragrant candles, plants and potpourris as many allergic people are sensitive to these odors. Apply perfume conservatively, as well.
- Don't burn wood in the fireplace. Smoke and ash can provoke breathing difficulty or an asthma attack. Also, request guests smoke cigarettes outdoors.

Find additional information on allergies and asthma, including allergy-safe holiday recipes, online at www.aaaai.org. To speak with a medical expert about preventing allergy and asthma symptoms, contact Kimberly Jahnke at kjahnke@aaaai.org.
The American Academy of Allergy, Asthma & Immunology (AAAAI) represents allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Established in 1943, the AAAAI is the largest professional medical association dedicated to the allergy/immunology specialty. The AAAAI has nearly 6,500 members in the United States, Canada and 60 other countries.

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, December 1, 2008

AAAAI: Long-lasting cold symptoms may be sinusitis

MILWAUKEE – If your stuffy nose and headache last for more than two weeks, it may be more serious than a cold. Winter is prime season for sinusitis, as the condition most often results from the common cold. Allergy sufferers are also more likely to develop sinusitis.
An estimated 31 million Americans develop sinusitis each year, leading to 18 million physician visits and $5.8 billion in overall health expenditures according to the American Academy of Allergy, Asthma & Immunology (AAAAI).
"Early on, the symptoms of colds and sinusitis are similar," said Anju Peters, MD, Chair of the AAAAI Rhinosinusitis Committee. "But if symptoms are worsening after 3-5 days, or if they are present for more than 10 days, then sinusitis is the likely culprit."
Sinusitis occurs when drains in the sinus cavities – hollow areas behind the forehead and cheeks – become blocked due to inflammation caused by a cold or allergies. The blockage prevents mucous from draining normally, leading to infection.
Sinusitis is easily recognized by a green or gray nasal discharge, foul tasting post-nasal drip, facial pain/pressure or light fever.
Sinusitis can last for months, or even years, if not properly treated. A physician will typically prescribe antibiotics to treat the infection and patients may also use decongestants to relieve stuffiness.
An allergist/immunologist is the best-qualified medical professional to diagnose and treat underlying allergies that contribute to sinusitis. Use the AAAAI Physician Referral Directory at www.aaaai.org to find an allergist/immunologist near you.
For more information about sinusitis and controlling allergies, visit www.aaaai.org.
The American Academy of Allergy, Asthma & Immunology (AAAAI) represents allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Established in 1943, the AAAAI has nearly 6,500 members in the United States, Canada and 60 other countries.

American Academy of Allergy Asthma & Immunology (AAAAI) - For immediate release, December 10, 2009

AAAAI clarifies appropriate use of LABAs

MILWAUKEE – A Food and Drug Administration advisory panel is set to meet tomorrow to discuss the future of a class of drugs often used to treat asthma.
The American Academy of Allergy, Asthma & Immunology (AAAAI) advises continued use of long-acting beta agonists (LABAs) with appropriate patients when prescribed as part of an asthma treatment plan.
The AAAAI believes that LABAs have a favorable risk/benefit ratio with proven positive clinical outcomes, as outlined in The National Asthma Education and Prevention Program Expert Panel Report 3 (EPR-3).
Asthma is one of the most common chronic health problems in the United States, impacting the lives of more than 20 million people and their families. Asthma causes airways within the lungs to tighten and swell which, in turn, restricts airflow and the supply of oxygen.

There are two primary forms of treatment for asthma. "Controllers," such as inhaled corticosteroids (ICS), reduce the inflammation, and "relievers," such as short-acting beta agonists (often called bronchodilators), treat the airway constrictions. Treating the underlying inflammation and relieving or preventing muscle contraction in the airways is necessary for most patients to control their asthma and prevent symptoms. Inhaled short-acting beta agonists should be taken only as needed and are not intended for daily use.

This approach to medication is affirmed in EPR-3. The report states that patients with persistent asthma (e.g., patients who have symptoms more than twice a week during the day or more than twice a month at night) need both long-term control medications to control asthma and prevent exacerbations, as well as quick relief medications for symptoms as needed. For many asthma patients, a daily treatment plan combining corticosteroid use with LABAs is recommended to control asthma symptoms and prevent life-threatening attacks. The guidelines were updated in 2007 to reflect the latest evidence on effectiveness and safety.

The addition of LABAs to inhaled corticosteroids has shown more favorable outcomes in controlling asthma than the use of higher doses of inhaled corticosteroids used alone, according to the majority of clinical trials. In fact, as the use of LABA inhalers has increased, the rate of asthma-related deaths has decreased.

The FDA's recent hearing has raised awareness of the drugs used to treat asthma and has generated some confusion among patients and their families. The AAAAI recommends contacting your allergist or primary care physician if you are concerned about your medication. For educational information about asthma or to find an allergist/immunologist in your area, visit www.aaaai.org.

The AAAAI represents allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Established in 1943, the AAAAI has nearly 6,500 members in the United States, Canada and 60 other countries.