edra | Issn 1764-1489 | Volume 49 N. 6/2017 – November 2017

European Annals of Allergy and Clinical Immunology

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

6/2017

European Annals of Allergy and Clinical Immunology

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

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

edra

Editors in Chief
Lorenzo Cecchi
Mário Morais-Almeida

Publishing Director
Nicola Miglino

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

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

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

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

Subscription
abbonamentiedra@lswr.it
Tel. 02 88184.317 - Fax 02 5656.1173
Italy subscription: 60 euro
World subscription: 85 euro
www.eurannallergyimm.com

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

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

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

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

Author Guidelines

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

Manuscript

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

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

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

Units and Abbreviations

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

References

References should be in the order:

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

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

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

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

Illustrations

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

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

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

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

Table of Contents

C. Chatain[1,2], I. Pin[2], P. Pralong[1], J.P. Jacquier[1], M.T. Leccia[1]

Medicinal bioactivites and allergenic properties of pumpkin seeds: review upon a pediatric food anaphylaxis case report

[1]Department of Dermatology, Allergology and Photobiology, Centre Hospitalier Universitaire de Grenoble, Grenoble, France
[2]Department of Pediatrics, Hôpital Couple Enfant, Centre Hospitalier Universitaire de Grenoble, Grenoble, France

Key words
allergens; child; food allergy; medicinal activity; pumpkin seed

Corresponding author
Catharina Chatain
Centre Hospitalier Universitaire de Grenoble
BP 217, 38043 Grenoble Cedex 09, France
Phone: +33 6 6707 8725
Fax: +33 4 7676 5558
E-mail: cchatain@chu-grenoble.fr

Doi
10.23822/EurAnnACI.1764-1489.19

Summary
Food allergy to pumpkin seed is considered very rare, and only some isolated case reports have so far been published. We report here a case of food anaphylaxis to pumpkin seed in an eight-year-old boy, who tolerated all other edible seeds, peanut and tree nuts, as well as pulp of different kinds of pumpkins and other fruits of the Cucurbitaceae family. From this observation, a review of the botanical, historical, medicinal and allergenic aspects of pumpkin and its seeds is proposed. With the advent of diets rich in omega-3 and omega-6 polyunsaturated fatty acids, edible seeds like pumpkin seed have been incorporated in the modern diet. Their incremental use in the food-processing industry might contribute to an increase in food allergy to pumpkin seed in the future.

Introduction

Comestible seeds like sesame, sunflower, flax, pumpkin and poppy seeds are increasingly incorporated in the modern diet because of their potential health benefits, associated with their high concentration in omega-3 and omega-6 polyunsaturated fatty acids, and with some of their biologically active components. Along with changing diet habits, hypersensitivity reactions to seeds are augmenting, but still are rarely suspected.
Pumpkin belongs to the *Cucurbitaceae* family. Its seeds have been used as traditional medicine in South Asia and both Americas. Since a few decades, as alternative medicine and healthy nutrition become increasingly popular, pumpkin seed has gained interest in the field of diet and disease research. Its various medicinal bioactivities, reviewed in this report, have contributed to qualify pumpkin seeds as an attractive nutriment, but hence also as a potential emerging allergen food source.

Food allergy to pumpkin seed is considered an extremely rare allergy. Only one case of severe allergy in a child after ingestion of pumpkin seeds has previously been reported in the literature (1). We describe another pediatric case of food anaphylaxis to pumpkin seed.

Materials and methods (case report)

An eight-year-old boy, with a history of allergic asthma to house dust mites, outgrown IgE-mediated food allergies to cow milk and cashew nut and persistent goat and sheep milk allergy, declared a grade 3 anaphylaxis with deep faintness, vomiting, facial and pharyngeal edema almost immediately after eating a German multigrain bread bun containing sunflower and pumpkin seeds. The parents treated the reaction with oral antihistamines and corticoids, but on worsening of symptoms during the following ten minutes did not hesitate to use the child's epineph-

rine auto-injector (Epipen®) with immediate efficacy. The boy was hospitalized for 24 hours in the nearby hospital.
At about 5 years of age, the child had already experienced an episode of faintness with nausea and oropharyngeal pruritus after ingestion of a bite of bread containing seeds, resolved rapidly after oral intake of antihistamines. This incident had therefore not been reported to the allergist before.
The child consumed without any problems the different cucurbits (different pumpkin and squash varieties, zucchini, cucumber and melon varieties including cantaloupe, canary melon and watermelon), peanuts and all kinds of nuts (almond, hazelnut, walnut, pecan nut, pistachio, cashew nut, Brazil nut, macadamia nut, coconut, pine kernel).

Results

We tested the pulp and the seeds of cultivars of the three *Cucurbita* species commonly found in Europe: C*ucurbita pepo, Cucurbita maxima, Cucurbita moschata.* In search of cross reactions, we tested also the pulp and the seeds of some cultivars of the two cucurbit genera *Cucumis* and *Citrullus*: three *Cucumis melo* or muskmelon varieties (cantaloupe, canary melon, honeydew), *Cucumis sativa* or cucumber, as well as *Citrullus lanatus* or watermelon. All kinds of other edible seeds and their oils (sesame, sunflower, flax, poppy), nuts, and flour types were tested for the same reason.
Skin prick-to-prick testing demonstrated a large positive 10 mm wheal and flare responses to different kinds of pumpkin seeds (**Figure 1** and **2**), *Cucurbita pepo* Lady Godiva, French *Cucurbita maxima* Rouge vif d'Estampes, French *Cucurbita moschata* Musquée de Provence, and a 5 mm wheal and flare response to *Cucurbita moschata* butternut squash seed, while the histamine control was 4 mm. Prick-to-prick skin tests with raw and cooked pulp of these pumpkin varieties were negative, as well as with immature zucchini (*Cucurbita pepo*) seeds, raw and cooked zucchini pulp and pumpkin seed oil.
Prick-to-prick tests were slightly positive with sunflower, flax and sesame seed, creating a 3.5 mm wheal. Poppy seed prick-to-prick tests were negative as well as prick-to-prick tests with linseed oil, sesame oil, rape seed oil (colza oil), cucumber, seeds and pulp of cantaloupe, honeydew, canary melon and watermelon.

Figure 1 *- Prick to prick test to pumpkin seed (C. pepo).*

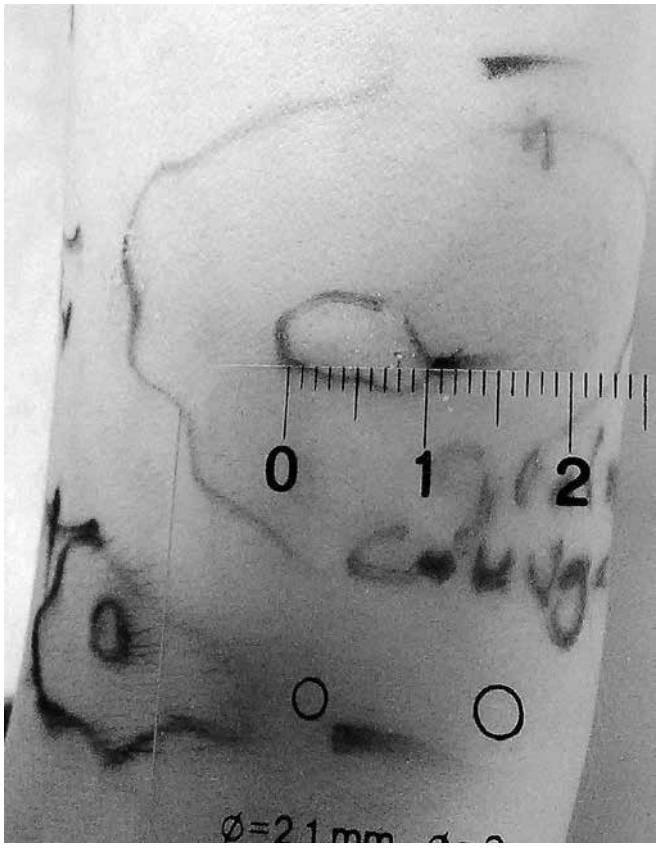


Figure 2 *- Prick to prick test to different pumpkin seeds.*

Prick-to-prick skin tests remained also negative to peanut, all kinds of nuts (almond, hazelnut, walnut, pecan nut, pistachio, cashew nut, Brazil nut, macadamia nut, coconut, pine kernel) and flours (wheat, rye, oat, soy, lupine, buckwheat, chestnut).
Specific IgE to pumpkin seed (*C. pepo*) were positive at 3.75 KU/L, while they remained negative to pumpkin pulp, as well as to sunflower seed, flaxseed, sesame seed and poppy seed. Furthermore, negative results were found for specific IgE to recombinant peach proteins pru p 1 (PR 10), pru p 3 (LTP), pru p 4 (profilin), peanut and hazelnut PR 10 (ara h 8 and cor a 1, respectively), peanut, hazelnut and walnut LTP (ara h 9, cor a 8 and jug r 3, respectively), peanut storage proteins ara h 1 (7S globulin or vicillin), ara h 2 (2S albumin) and ara h 3 (11S globulin or legumin), cashew nut storage protein ana o 3 (2S albumin), walnut storage protein jug r 1 (2S albumin), and hazelnut storage protein cor a 14 (2S albumin), whereas specific IgE to hazelnut storage protein cor a 9 (11S globulin) were slightly positive at 0.13 KU/L (by ImmunoCAP® Thermo Fisher Scientific, Phadia AB Uppsala Sweden). Total serum IgE measured 212 KUI/L, and serum baseline tryptase 8.06 µg/L.
An oral challenge test with a seed mix (sunflower, flax, sesame and poppy seed) was proposed on account of the cutaneous sensitization to these seeds, and remained negative at significant doses (cumulative dose of 20 g).

Discussion

Botanical aspects

Cucurbitaceae, also called cucurbits, are herbaceous climbers or woody lianas grouped together in a plant family consisting of about 95 genera and 950-80 species (2). The *Cucurbitaceae* family ranks among the highest of plant families for number and percentage of species used as human food. Edible genera include:

- *Cucurbita*, squash, pumpkin, zucchini, some gourds
- *Citrullus*, watermelon (*C. lanatus*), *C. colocynthis*
- *Cucumis*, cucumber (*C. sativus*), various melons (*C. melo).*
- Five *cucurbita* species have been domesticated:
- *Cucurbita argyrosperma* Huber, e.g. Mexican cushaw pumpkin
- *C. ficifolia* Bouché, e.g. South American black seed gourd, known also as fig leaf squash or Malabar gourd
- *C. maxima* Duchesne ex Poiret, some cultivars: all giant pumpkins, Rouge Vif d'Estampes, Red kuri squash also known as Hokkaido squash, French turban squash, Buttercup squash, Hubbard squash
- *C. moschata* (Duchesne ex Lam.) Duchesne ex Poiret, some cultivars: Musquée de Provence, Butternut squash, Crookneck type squash, Banana Squash
- *C. pepo* L., some cultivars: pumpkin, Acorn squash also known as Des Moines Squash or Pepper Squash, Spaghetti squash, Straightneck squash, zucchini (3,4).

Species of cucurbits are cultivated in most countries of the world, where crop plants can be grown in the summer at warm temperatures. All species are sensitive to frost. Most of the plants in this family are annual creeping or climbing plants (vines), with five vigorous stems and five-lobed or palmately divided leaves with long petioles. The leaves are alternately arranged on the stem. The stems are hairy and pentangular, and have spring-like tendrils. Many species have large, yellow or white flowers with five fused petals. The flowers are unisexual, with male and female flowers on different plants (dioecious) or on the same plant (monoecious). The large and fleshy fruit, usually with a hard outer covering, is often a kind of modified berry called a pepo. The seeds are ovate-elliptical, flattened, 15 - 25 x 7 - 12 mm, of dark brown to black or creamy white color (2-6).

Historical aspects

Cucurbitaceae probably evolved in the Late Cretaceous, some 60 million years ago (2). The varieties of pumpkin and squash originated in various places throughout the Americas, specifically central Mexico, Peru and the Eastern side of the United States. Archaeological records suggest that *Cucurbita* species were one of the first plants to be domesticated (3,5,7). Cultivation by the inhabitants of Guila Naquitz cave date between 10,000 and 8,000 BC, predating corn and beans by more than 4,000 years (8). *Cucurbita* species were brought to Europe after Columbus' exploration after 1492 (5). *Cucumis* and *Citrullus* species originated in Africa and Western Asia (India). Finds in Egyptian tombs dating from the 16th to the 12th century BC have revealed that sweet cucurbits were being eaten by pharaohs, as well as later on by Roman and Byzantine empires from the 2nd to the 6th centuries (3,5,9).
In Austria and adjacent countries, pumpkins have been grown for production of oil for about three centuries. Because the seed coat comprises about 20% or more of the seed weight, new technologies were sought to utilize in oil seed pumpkins. At about the turn of the 20th century, a shell-less seed variant was discovered and subsequently bred because of the greater efficiency in oil recovery, since the seeds did not have to be laboriously hand shelled (10).
In the last two decades, pumpkin seed has been the focus of increased interest in the field of diet and disease research due to the potential health benefits associated with some of its biologically active components. With the advent of diets rich in omega-3 and omega-6 polyunsaturated fatty acids, pumpkin seeds and other seeds have been incorporated in the modern diet. Pumpkin seeds are used as additives to confectionery and bakery products or eaten as snacks after being salted and roasted. Pumpkin seed oil (especially that obtained from hull-less seeds) is utilized by both pharmaceutical and food industries (in the latter generally as salad oil) (11). Besides its use as food, pumpkinseed flour is used by fishermen for baits to lure fish (12).

Phytochemistry of pumpkin seeds

Pumpkin (*Cucurbita sp.*) seeds are a good source of essential micro-elements such as K, Na, Cr, Mg, Zn, Cu, Mo and Se. Seeds are valued for their high protein amount (30 - 37%) with presence of all 9 essential amino acids, as well as for their high quantity of lipids (40 - 50%) containing essential fatty acids, especially alpha linoleic acid (omega-6). The lipid fraction of pumpkin seeds encloses also high concentrations of bioactive components: phytosterols, carotenoids, tocopherols and phenolic compounds (6,10,11,13,14). Fat-soluble tocopherols and carotenoids, as well as phytosterols and polyphenols, have antioxidant activity and play an important role in decreasing DNA damage, diminishing lipid peroxidation, maintaining immune function, and inhibiting malignant transformation or proliferation (11,13-15).

Medicinal bioactivities of pumpkin seeds

The seeds of *Cucurbita sp.* have been traditionally used as medicine in China, India, Korea, Yugoslavia, Argentina, Brazil, Mexico and America, to treat intestinal parasites, urinary infections, bladder and kidney stones, biliary vesicle and prostate problems. Due to their anti-inflammatory and anti-oxidative properties, pumpkin seeds are thought to slow the aging process, reduce the risk of cataract development, promote wound healing and reduce symptoms of inflammatory diseases such as arthritis (6,10,14-16). Broadspectrum antibacterial and antifungal activity has been reported for different peptides found in pumpkin seed oil, such as α- and β-moschins (MW: 12 kDa), MAP28 (MW: 28 kDa), MAP2 (MW: 2249D), MAP4 (MW: 4650D), MAP11 (MW: 11696D), peptide (MW: 8 kDa), and pumpkin albumin 2S (6,10,17). Curcubitin and the protoberberine alkaloids berberine and palmatine have shown anthelminthic activity (18), which was reported at the minimum inhibitory concentration of 23 g of pumpkin seed in 100 ml of distilled water in preclinical studies (10).

Because of their antimutagenic and immunomodulatory activity, pumpkin seeds have also been associated with lower risk of gastric, breast, lung, prostate and colorectal cancers (6,17,19,20). The ribosome-inactivating proteins Moschatin and Cucurmosin from the mature seeds of pumpkin (*C. moschata*) were reported to inhibit melanoma cell proliferation as well as leukemia, lung adenocarcinoma and pancreas cancer cell proliferation for the latter (21-23). Pumpkin albumin 2 S was shown to exhibit a strong anticancer activity toward breast adenocarcinoma (MCF-7), ovarian teratocarcinoma (PA-1), prostate cancer (PC-3 and DU-145) and hepatocellular carcinoma (HepG2) cell lines (17).

Rich in omega-3 and omega-6 fatty acids, phytosterols and secoisolariciresinol, a lignan acting as phytoestrogen and anti-oxidant, pumpkin seeds were shown to have protective activity against cardiovascular diseases and non-alcoholic fatty liver disease, by improving plasma lipid profile, lowering blood pressure and attenuating arthrosclerosis development (24-26). Bioactive macromolecules, such as Tocopherol, Trigonelline, Nicotinic acid and D-chiro-inositol, found in pumpkin seeds, possess hypoglycemic properties and could assist in maintaining glycemic control (27,28).

Because of their high β-sitosterol content, pumpkin seeds (*C. pepo*) are further used to treat benign prostatic hyperplasia (29) and overactive bladder (30), especially in German speaking countries where pumpkin seed is officially, since a few decades, in the German Pharmacopeia (Granu Fink®) (31).

Finally, because of the high tryptophan content, pumpkin seeds might ease depression, anxiety, nervous irritability and insomnia (6,32,33). Furthermore, they have shown to improve the iron status (34) and have blood-coagulatory effects (6,35). Their multiflorane-type triterpenes have melanogenesis inhibitory activity and thus may be potential skin whitening agents (36). Pumpkin seed oil has shown to be effective for the treatment of hair growth in male patients with mild to moderate androgenetic alopecia (37). **Table I** summarizes the medicinal and pharmacological effects of *Cucurbita sp.* seeds.

Allergenicity

Allergy to pumpkin is considered a very rare disorder and so far only a few isolated case reports have been published. All parts of the fruit can be responsible for allergic symptoms.

Cucurbita sp. peel

Cucurbita sp. peel can cause allergic contact dermatitis (38,39) after peeling and cutting-up uncooked *Cucurbita* species. (*C. maxima* varieties and butternut squash, respectively). Arochena et al. report a case of contact urticaria and angioedema of the face after handling onion and zucchini (*C. pepo*) with no symptoms after ingestion of cooked zucchini, for which an IgE-based mechanism was demonstrated (40). By analogy to the major allergen of melon peel (*Cucumis melo*), LTP could be one possible allergen for contact allergy to pumpkin (41).

Curcurbita sp. pulp

In 2000, Figuendo et al. publish the first case of immediate-type systemic reaction associating pharyngeal and generalized itching, angioedema of the face and mild dyspnoea after ingestion of pumpkin pulp (*C. pepo*) in a 28-year-old woman, with cross reactivity to cucumber, zucchini, muskmelon and watermelon, supported by positive prick-to-prick tests and positive challenge tests (42). In the same year, Reindl et al. report four cases of food allergy to raw and cooked zucchini (*C. pepo*), ranging from oral allergy syndrome to grade two anaphylaxis with digestive symp-

Table I *- Important bioactive compounds from pumpkin seeds and their biological activities.*

Specialty	Substance	Indications
Infectiology	moschin, MAP 2, MAP 4, MAP11, MAP28, peptide (MW: 8 kDa), albumin 2S	bacterial and fungal infections of the skin, urinary tract and intestine
	cucurbitin, berberine, palmatine	intestinal helminthic infections
Rheumatology	anti-inflammatory compounds	inflammatory diseases, arthritis
Gastroenterology	hydroxyl pentacyclic triterpene acids	biliary vesicle problems
	fiber	constipation
Dermatology	linoleic acid, tocopherols,	wound healing
	phytosterols	alopecia
	multiflorane-type triterpene esters	hyperpigmentation disorders (melanogenesis inhibition)
Ophthalmology	carotenoids	cataract
Psychology	tryptophan	depression, anxiety, nervous irritability, insomnia
Neurology	carotenoids, tocopherols	mental and physical ageing
Gynecology	phytosterols	lactation, postpartum edema
Nephrology, Urology, Andrology	phytosterols (β-sitosterol, delta-7-sterols), lignans, carotenoids, tocopherols, omega 3 and 6 fatty acids	benign prostatic hyperplasia, bladder and kidney stones, irritable bladder, enuresis nocturna, impotence
Hematology	coumaric acid	blood coagulation
Cardiovascular Disease, Metabolic Syndrome	polyphenols, lignans, phytosterols, omega-3 and omega-6 fatty acids	hypertension, hypercholesterolemia, dyslipidemia, atherosclerosis, non-alcoholic fatty liver disease
Diabetology	trigonelline, nicotinic acid, D-chiro-inositol, tocopherol, lignans	diabetes, hyperglycemia
Oncology	carotenoids, polyphenols, squalene, phytosterols, lignans	gastric, breast, lung, prostate and colorectal cancers
	pumpkin 2S albumin	breast adenocarcinoma (MCF-7), ovarian teratocarcinoma (PA-1), prostate cancer (PC-3 and DU-145) and hepatocellular carcinoma (HepG2) cells
	moschatin	melanoma
	cucurmosin, MAP2, MAP4	melanoma, leukemia, lung adenocarcinoma and pancreas cancer

toms or stridor (43). The first case of pediatric food anaphylaxis to pumpkin pulp (*C. maxima*) was published by Hagendorens in 2009 (44). Pumpkin can also be responsible for food-dependent exercise-induced anaphylaxis (45) or food protein-induced enterocolitis syndrome (46, 47).

Cucurbita sp. allergy can be isolated (43[three of the four cases]-46,48,49), but in some cases is correlated with clinical cross-allergenicity to other fruits of the cucurbitaceous family (cucumber, melon, and/or watermelon) (42,43,50). Pumpkin pulp allergy can be part of the latex-fruit syndrome (51). Finally, it may be associated with sensitization to peach (50,52).

For *Cucurbita sp.* pulp allergy, possible allergens could be profilin (15 kDa protein, Cuc p 2, Cuc ma 2), LTP (8.9 kDa protein), cross-reacting carbohydrate determinants and zucchini-specific proteins of molecular weight of 16-17 kDa and of 41.5 kDa (42,43,48,49). González De Olano et al. identify an 18 kDa protein, corresponding to cyclophilin (Cuc ma CyP) as a relevant allergen for oral allergy syndrome to pumpkin (50).

Cucurbita sp. seed

Only seven cases of food allergy after ingestion of pumpkin seeds have so far been reported, with one of them also presenting occupational contact urticaria to pumpkin seeds. All six subjects tolerated pumpkin pulp and other fruits of the cucurbitaceous family (cucumber, melon and/or watermelon) (1,12,53-55).

Fritsch et al. describe three cases of adult fishermen who experienced allergic symptoms immediately after ingestion of food containing pumpkin seed. The clinical symptoms ranged from oropharyngeal itching and swelling to bronchial asthma. The patients had previously been sensitized by inhalation of a pumpkinseed flour mixture in its powdered form during preparation of baits to lure fish (the baits were made of a mixture of corn bran, wheat bran, pumpkinseed flour and water and then molded into balls) (12).

Baur et al. report the particular case of an 18-year-old man with anaphylaxis associating pharyngeal swelling and dysphagia after eating rolls with pumpkin seed who three years later, when working as a baker, developed occupational contact urticaria to pumpkin seed (53).

Rodríguez-Jiménez record anaphylaxis with edema and erythema accompanied by a sensation of dyspnoea after ingestion of toasted pumpkin seeds (*C. maxima* species) in a 33-year-old pollen-allergic man who reported also facial edema and erythema after eating peach, apple, unpeeled pear, and orange (54).

Caubert et al. publish the first pediatric case of an 11-year-old boy with history of fish allergy and atopic dermatitis, who presented anaphylaxis with urticaria, angioedema and asthma after eating pumpkin seeds (1).

In a very recent report, Doll et al. describe a case of anaphylaxis in a 70-year-old woman with history of angioedema to shellfish. Almost immediately after ingestion of pumpkin seeds included in a trail mix, symptoms initiated with nausea and sense of impending doom and were followed by several syncopal episodes despite administration of an expired Epipen® (55). In accordance with Prieto-Gracia et al. (56) who describe two cases of food anaphylaxis with underlying mast cell disorder, the severity of the reaction (several syncopal episodes), the absence of cutaneous symptoms, especially hives, and a REMA score of ≥ 2 might be suggestive of an underlying clonal mast cell order in Doll and al.'s patient, despite a normal baseline serum tryptase (55).

Set apart from these cases of food allergy to *Cucurbita* sp. seed, but worth including in our review, is a report of food allergy to egusi-itoo seeds (seeds of African pumpkin *Cucumeropsis mannii*), a species of another *Cucurbitaceae* genus, *Cucumeropsis*. An 8-year-old Togolese girl presented an angioedema of the face after ingestion of egusi-itoo seeds, with no cross-allergy to pumpkin seeds of the *Cucurbita* species. A 60 kDa protein, specific to African pumpkin seed, was identified (57).

Pumpkin seed sensitivity may be associated with allergy to other seeds as reported by Lavine et al. in a 3-year-old boy allergic to sunflower and poppy seeds (58).

The allergens of pumpkin seed have not been well characterized. Immunoblot studies on the sera of the three patients described by Fritsch et al. revealed a 14 kDa protein which is probably a homologue of profilin, as it was completely inhibited by recombinant birch profilin. Besides the 14 kDa protein, proteins of molecular weight of 13, 36, 48, 69, 77, and 87 kDA were detected (12). Rodríguez-Jiménez identified a 12 kDa protein which however was not homologous to the lipid transfer protein of peach (pru p3), although the patient's clinical symptoms (concomitant allergy to peach, apple, unpeeled pear, and orange) suggested involvement of an LTP (54).

Potential allergens of edible seeds may correspond to storage proteins which are found in the seeds of a wide range of mono- and di-cotyledonous plants. Their hydrolysis provides necessary amino acids during germination and to the growing plant. The storage proteins most abundantly found in seeds are cupins or salino-soluble globulins, to which belong 7S globulins or vicilins and 11S globulins or legumins. Other storage proteins are 2S albumins. Their compact structure, their important size and abundance in the seeds, as well as their resistance to heat denaturation and to hydrolysis by digestive proteases are thought to be responsible for their important allergenic properties (58,59). Cupins (7S vicilins and 11S legumins) have been identified in sesame seed, and 2S albumin storage proteins in both sesame and sunflower seed (60). The important sequential and structural homology of storage proteins of different origins may be responsible for cross-reactivity between different edible seeds, peanuts and tree nuts (58-60).

In our case, no clinical cross-allergenicity has been demonstrated. However, slightly positive IgE to hazelnut 11S globulin (cor a 9) might be suggestive of biological cross-reactivity and implication of pumpkin specific 11S globulin in the boy's anaphylactic reaction.

Conclusions

Because of their potential therapeutic effects in the prevention of cardiovascular disease, cancer and aging process, seeds like pumpkin seeds are increasingly included in many foods, in alternative or natural medicines and in cosmetics. Pumpkin seed food allergy may therefore be expected to augment in the future. It should be assessed by carefully taking the patient's medical history and by including common edible seeds in food allergy testing panels (e.g. sesame, sunflower, pumpkin, flax and poppy seed).

References

1. Caubet JC, Hofer MF, Eigenmann PA, Wassenberg J. Snack seeds allergy in children. Allergy 2010; 65(1):136-7.
2. Schaefer H, Renner SS. Phylogenetic Relationships in the Order Cucurbitales and a New Classification of the Gourd Family (Cucurbitaceae). Taxon 2011; 60(1):122-38.

3. Kumar SR. Cucurbits: History, Nomenclature, Taxonomy, and Reproductive Growth. in Handbook of Cucurbits: Growth, Cultural Practices, and Physiology. Mohammad Pessarakli CRC Press. 2016:3-21
4. https://hort.purdue.edu/newcrop/1492/cucurbits.html
5. http://academics.hamilton.edu/foodforthought/our_research_files/cucurbitaceae.pdf
6. Yadav M, Jain S, Tomar R, Prasad GB, Yadav H. Medicinal and biological potential of pumpkin: an updated review. Nutr Res Rev 2010; 23(2):184-90.
7. Bisognin DA. Origin and Evolution of cultivated Cucurbits. Ciência Rural 2002; 32(5):715-23.
8. Smith BD. The initial domestication of C. pepo in the Anerucas 10,000 years ago. Science 1997; 276(5314):932-4.
9. Janick J, Paris HS, Parrish DC. The cucurbits of Mediterranean antiquity: identification of taxa from ancient images and descriptions. Ann Bot 2007; 100(7):1441-57.
10. Caili F, Huan S, Quanhong L. A review on pharmacological activities and utilization technologies of pumpkin. Plant Foods Hum Nutr 2006; 61(2):73-80.
11. Nawirska-Olszańska A, Kita A, Biesiada A, Sokół-Łętowska A, Kucharska AZ. Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars. Food Chem 2013; 139(1-4):155-61.
12. Fritsch R, Ebner H, Kraft D, Ebner C. Food allergy to pumpkin-seed-characterization of allergens. Allergy 1997; 52(3):335-7.
13. Kalogeropoulos N, Chiou A, Ioannou MS, Karathanos VT. Nutritional evaluation and health promoting activities of nuts and seeds cultivated in Greece. Int J Food Sci Nutr 2013; 64(6):757-67.
14. Kim MY, Kim EJ, Kim YN, Choi C, Lee BH. Comparison of the chemical compositions and nutritive values of various pumpkin (Cucurbitaceae) species and parts. Nutr Res Pract 2012; 6(1):21-7.
15. Veronezi CM, Jorge N. Bioactive compounds in lipid fractions of pumpkin (Cucurbita sp) seeds for use in food. J Food Sci 2012; 77(6):C653-7.
16. Bardaa S, Ben Halima N, Aloui F, Ben Mansour R, Jabeur H, Bouaziz M, Sahnoun Z. Oil from pumpkin (Cucurbita pepo L.) seeds: evaluation of its functional properties on wound healing in rats. Lipids Health Dis 2016; 15:73.
17. Tomar PP, Nikhil K, Singh A, Selvakumar P, Roy P, Sharma AK. Characterization of anticancer, DNase and antifungal activity of pumpkin 2S albumin. Biochem Biophys Res Commun 2014; 448(4):349-54.
18. Grzybek M, Kukula-Koch W, Strachecka A, Jaworska A, Phiri AM, Paleolog J, Tomczuk K. Evaluation of anthelmintic activity and composition of pumpkin (Cucurbita pepo L.) seed extracts-in vitro and in vivo studies. Int J Mol Sci 2016; 17(9) pii: E1456.
19. Medjakovic S, Hobiger S, Ardjomand-Woelkart K, Bucar F, Jungbauer A. Pumpkin seed extract: cell growth inhibition of hyperplastic and cancer cells, independent of steroid hormone receptors. Fitoterapia 2016; 110:150-6.
20. Richter D, Abarzua S, Chrobak M, Vrekoussis T, Weissenbacher T, Kuhn C, Schulze S, Kupka MS, Friese K, Briese V, Piechulla B, Makrigiannakis A, Jeschke U, Dian D. Effects of phytoestrogen extracts isolated from pumpkin seeds on estradiol production and ER/PR expression in breast cancer and trophoblast tumor cells. Nutr Cancer 2013; 65(5):739-45.
21. Xia HC, Li F, Li Z. Purification and characterization of moschatin, a novel type I ribosome-inactivating protein from the mature seeds of pumpkin (Cucurbita moschata), and preparation of its immunotoxin against human melanoma cells. Cell Res 2003; 13(5):369-374.
22. Hou X, Meehan EJ, Xie J, Huang M, Chen M, Chen L. Atomic resolution structure of cucurmosin, a novel type 1 ribosome-inactivating protein from the sarcocarp of Cucurbita moschata. J Struct Biol 2008; 164(1):81-7.
23. Zhang B, Huang H, Xie J, Xu C, Chen M, Wang C, Yang A, Yin Q. Cucurmosin induces apoptosis of BxPC-3 human pancreatic cancer cells via inactivation of the EGFR signaling pathway. Oncol Rep 2012; 27(3):891-7.
24. Morrison MC, Mulder P, Stavro PM, Suárez M, Arola-Arnal A, van Duyvenvoorde W, Kooistra T, Wielinga PY, Kleemann R. Replacement of dietary saturated fat by PUFA-Rich pumpkin seed oil attenuates non-alcoholic fatty liver disease and atherosclerosis development, with additional health effects of virgin over refined oil. PLoS One 2015; 10(9):e0139196.
25. Gossell-Williams M, Lyttle K, Clarke T, Gardner M, Simon O. Supplementation with pumpkin seed oil improves plasma lipid profile and cardiovascular outcomes of female non-ovariectomized and ovariectomized Sprague-Dawley rats. Phytother Res 2008; 22(7):873-7.
26. El-Mosallamy AE, Sleem AA, Abdel-Salam OM, Shaffie N, Kenawy SA. Antihypertensive and cardioprotective effects of pumpkin seed oil. J Med Food 2012; 15(2):180-9.
27. Adams GG, Imran S, Wang S, Mohammad A, Kok MS, Gray DA, Channell GA, Harding SE. The hypoglycemic effect of pumpkin seeds, Trigonelline (TRG), Nicotinic acid (NA), and D-Chiro-inositol (DCI) in controlling glycemic levels in diabetes mellitus. Crit Rev Food Sci Nutr 2014; 54(10):1322-9.
28. Bharti SK, Kumar A, Sharma NK, Prakash O, Jaiswal SK, Krishnan S, Gupta AK, Kumar A. Tocopherol from seeds of Cucurbita pepo against diabetes: validation by in vivo experiments supported by computational docking. J Formos Med Assoc 2013; 112(11):676-90.
29. Damiano R, Cai T, Fornara P, Franzese CA, Leonardi R, Mirone V. The role of Cucurbita pepo in the management of patients affected by lower urinary tract symptoms due to benign prostatic hyperplasia: a narrative review. Arch Ital Urol Androl 2016; 88(2):136-43.
30. Nishimura M, Ohkawara T, Sato H, Takeda H, Nishihira J. Pumpkin seed oil extracted from Cucurbita maxima improves urinary disorder in human overactive bladder. J Tradit Complement Med 2014; 4(1):72-4.
31. https://online.rote-liste.de/suche/Granu%20fink
32. Hudson C, Hudson S, MacKenzie J. Protein-source tryptophan as an efficacious treatment for social anxiety disorder: a pilot study. Can J Physiol Pharmacol 2007; 85(9):928-32.
33. Hudson C, Hudson SP, Hecht T, MacKenzie J. Protein source tryptophan versus pharmaceutical grade tryptophan as an efficacious treatment for chronic insomnia. Nutr Neurosci 2005; 8(2):121-7.
34. Naghii MR, Mofid M. Impact of daily consumption of iron fortified ready-to-eat cereal and pumpkin seed kernels (Cucurbita pepo) on serum iron in adult women. Biofactors 2007; 30(1):19-26.
35. Krishnamoorthi R, Gong YX, Richardson M. A new protein inhibitor of trypsin and activated Hageman factor from pumpkin (Cucurbita maxima) seeds. FEBS Lett 1990; 273(1-2):163-7.
36. Kikuchi T, Ueda S, Kanazawa J, Naoe H, Yamada T, Tanaka R. Three new triterpene esters from pumpkin (Cucurbita maxima) seeds. Molecules 2014; 19(4):4802-13.
37. Cho YH, Lee SY, Jeong DW, Choi EJ, Kim YJ, Lee JG, Yi YH, Cha HS. Effect of pumpkin seed oil on hair growth in men

with androgenetic alopecia: a randomized, double-blind, placebo-controlled trial. Evid Based Complement Alternat Med 2014; 2014:549721.
38. Sinha S, Pasricha J, Sharma R, Kandhari K. Vegetables responsible for contact dermatitis of the hands. Arch Dermatol 1977; 113(6):776-9.
39. Potter TS, Hashimoto K. Butternut squash (Cucurbita moschata) dermatitis. Contact Dermatitis 1994; 30(2):123.
40. Arochena L, Gámez C, del Pozo V, Fernández-Nieto M. Cutaneous allergy at the supermarket. J Investig Allergol Clin Immunol 2012; 22(6):441-2.
41. Gandolfo-Cano M, Bartra J, González-Mancebo E, Feo-Brito F, Gómez E, Bartolomé B, Muñoz-García E, Sanz Maroto A, Vivanco F, Cuesta-Herranz J, Pastor-Vargas C. Molecular characterization of contact urticaria in patients with melon allergy. Br J Dermatol 2014; 170(3):651-6.
42. Figueredo E, Cuesta-Herranz J, Minguez A, Vidarte L, Pastor C, De Las Heras M, Vivanco F, Lahoz C. Allergy to pumpkin and crossreactivity to other Cucurbitaceae fruits. J Allergy Clin Immunol 2000; 106(2):402-3.
43. Reindl J, Anliker MD, Karamloo F, Vieths S, Wüthrich B. Allergy caused by ingestion of zucchini (Cucurbita pepo): characterization of allergens and cross-reactivity to pollen and other foods. J Allergy Clin Immunol 2000; 106(2):379-85.
44. Hagendorens MM, Carrette M, Bridts CH, Stevens WJ, Ebo DG. Allergy from giant pumpkin (Cucurbita maxima) is not a fairy tale. Allergy 2009; 64(11):1694-6.
45. Kim SM, Yoo SH, Kim MK. A Case of Squash-dependent Exercise-induced Anaphylaxis. Korean J Asthma Allergy Clin Immunol 2011; 31(2):140-3.
46. Nowak-Wegrzyn A, Sampson HA, Wood RA, Sicherer SH. Food protein-induced enterocolitis syndrome caused by solid food proteins. Pediatrics 2003; 111(4 Pt 1):829-35.
47. Ruffner MA, Finegold DN, MacGinnitie AJ. Infant with unusual food reactions. Acta Paediatr 2011; 100(10):1289,1394-5.
48. Damiani E, Aloia AM, Priore MG, Nardulli S, Macchia L, Ferrannini A. Zucchini allergy: a case report. Rev Fr Allergol 2011; 51(5):515-6.
49. Asero R, Mistrello G, Amato S. A case of allergy to zucchini. Eur Ann Allergy Clin Immunol 2012; 44(5):205-6.
50. González De Olano D, González-Mancebo E, Macadán SS, Cano MG, Pérez-Gordo M, Ortega BC, Vivanco F, Vargas CP. Allergy to pumpkin with cyclophilin as the relevant allergen. Ann Allergy Asthma Immunol 2010; 104(1):98-9.
51. Pereira C, Tavares B, Loureiro G, Lundberg M, Chieira C. Turnip and zucchini: new foods in the latex-fruit syndrome. Allergy 2007; 62(4):452-3.
52. La Shell MS, Otto HF, Whisman BA, Waibel KH, White AA, Calabria CW. Allergy to pumpkin and crossreactivity to pollens and other foods. Ann Allergy Asthma Immunol 2010; 104(2):178-80.
53. Baur X, Gahnz G. Allergy to pumpkin seed in the form of intolerance and Occupational contact urticaria: a case report. Dermatologie in Beruf und Umwelt 2002; 50(5):178-9.
54. Rodríguez-Jiménez B, Domínguez-Ortega J, Ledesma A, González-García JM, Kindelan-Recarte C. Food allergy to pumpkin seed. Allergol Immunopathol (Madr) 2010; 38(1):50-1.
55. Doll R, Johnson J, Peppers BP, Tcheurekdjian H, Hostoffer R. IgE-mediated anaphylactic shock caused by pumpkin seed in an adult. Ann Allergy Asthma Immunol 2017; pii: S1081-1206(16)31394-1.
56. Prieto-García A, Álvarez-Perea A, Matito A, Sánchez-Muñoz L, Morgado JM, Escribano L, Álvarez-Twose I. Systemic mastocytosis presenting as IgE-mediated food-induced anaphylaxis: a report of two cases. J Allergy Clin Immunol Pract 2015; 3(3):456-8.
57. Zana H, Moneret Vautrin DA, Guérin L, Kanny G, Leduc V. Allergie alimentaire isolée aux graines de courge africaine. Rev Fr Allergol 2005; 45(3):275.
58. Lavine E, Ben-Shoshan M. Allergy to sunflower seed and sunflower butter as proposed vehicle for sensitization. Allergy Asthma Clin Immunol 2015; 11(1):1-3.
59. Rougé P, Brunet E, Borges JP, Jauneau A, Saggio B, Bourrier T, Rancé F, Didier A, Barre A. Proteins with cupin motif as major seed allergens. Rev Fr Allergol 2011; 51(1):36-40.
60. Patel A, Bahna SL. Hypersensitivities to sesame and other common edible seeds. Allergy 2016; 71(10):1405-13.

D. El-Qutob[1]

Shrimp allergy: beyond avoidance diet

[1]Unit of Allergy, University Hospital of La Plana, Vila-Real, Spain

Key words
shrimp; allergy; food; hypersensitivity; immunotherapy; vaccine; tropomyosin

Corresponding author
David El-Qutob
Unit of Allergy University Hospital of La Plana
Carretera Vila-Real-Burriana Km 0.5
Vila-Real (Castellon) 12540, Spain
Phone: +34 964 3999 61
Fax: +34 964 3576 01
E-mail: elqutob@comv.es

Doi
10.23822/EurAnnACI.1764-1489.16

Summary
Currently, the management of people diagnosed with shellfish allergy relies on the avoidance of those foods. HDM immunotherapy has been reported to induce both shrimp allergy in non-allergic patients, and shrimp tolerance in shrimp-allergic patients. This article summarizes therapeutic options other than avoidance diet for shrimp allergic patients available once the diagnostic is established, such as production of hypoallergenic shrimp, use of immunotherapy with modified allergens, probiotics and Chinese herbal formulations.

List of abbreviations/acronyms
House dust mites (HDM)
Specific Immunoglobulin E (sIgE)
Allergen-specific immunotherapy (ASIT)
Sublingual immunotherapy (SLIT)
Chinese herbal formulations (CHF)

Introduction

Popularly, "shellfish" and "seafood" are often used interchangeably, but they mean different things. "Seafood" is a general term that refers to any edible aquatic animals, whereas "shellfish" refers to invertebrate aquatic animals usually fitted with a rigid exoskeleton (crustaceans and mollusks), edible, and likely to be traded for human consumption (1). Crustaceans are classified among arthropods together with arachnids and insects, whereas mollusks include bivalves, gastropods and cephalopods (2). In the last years, shellfish consumption has increased in popularity and frequency worldwide. This growing demand for shellfish, with an increment of their extraction and cultivation, has been accompanied by increasing reports of adverse reactions, many of which of immune mechanism, produced by ingestion or manipulation, affecting both consumers and seafood workers (3-5). Shellfish is one of the leading causes of food allergy, with a prevalence of 2.8-8% among all food allergies (6,7), and it is a common cause of food-induced anaphylaxis (8,9). Cases of shrimp allergy-induced exercise and NSAID-dependent anaphylaxis are described (10,11). Shellfish allergy is much less frequent in children than in adults (12). Anaphylactic reactions are more frequent in young people, atopic individuals and asthmatics (13). The crustaceans (shrimp, lobster and crab) are the main cause of shellfish allergy. Usually, subjects with shrimp hypersensitivity react clinically to other types of crustaceans. Shrimp is the most studied crustacean from an allergenic point of view.

Allergens

There are no differences between the protein bands from the extracts of boiled shrimp water and boiled shrimp (14). However, there are differences between extracts from cooked and raw shrimp (15).
Tropomyosin (Pen a 1), a protein from muscle, has been the first shrimp allergen detected (16). It is a major allergen of 38-41 kDa, and it is responsible for cross-reactivity between members of the shellfish family, particularly among the crustaceans. This led to its definition as an invertebrate panallergen (17). Tropomyosin has also been defined as a major allergen in other crustaceans (18), with very high homologies, up to 98%, among crustacean species including crawfish, crab and lobster (19,20). Tropomyosin is a panallergen responsible for the cross-reactivity between the members of the Arthropoda class, including shrimp (Pen a 1) and house dust mites (HDM) (Der p1 10, Der f 10). The prevalence of shrimp allergy is higher in regions with high prevalence of HDM allergy, such as Canarian Islands (21). In fact, almost all patients sensitized to shrimp showed positive skin prick test to HDM with/without clinical relevance, and 20-29% of HDM allergic patients showed sensitization to Der p 10 (22), tropomyosin from the HDM *Dermatophagoides pteronyssinus*. Frequently, HDM allergic patients are sensitized to shellfish with food tolerance. It is thought that inhaled tropomyosins from HDM are the primary sensitizer for shellfish allergy in warm and humid tropical climates (23).
There is also an important cross-reactivity with HDM Der p 10 and tropomyosin Pen a 1. There is not cross-reactivity between tropomyosins from filogenetically distant species. Therefore, tropomyosin from vertebrates is not allergenic.
Other minor allergens have been identified and characterized in shrimp. Arginine kinase is a 40 kDa allergen (Pen m 2) from muscle identified in shrimp (24,25) but also in mollusks (26). It was recognized in 27% out of a group of shrimp allergic subjects (25). In 2008, Ayuso et al. identified in Pacific white shrimp (*Litopenaeus vannamei*), a might light chain (Lit v 3) with high similarity to Bla g 8, a cockroach myosin light chain (27). Immunoblotting demonstrated immunoglobulin E (IgE) binding to a 20-kDa shrimp protein in 21 (55%) out of 38 sera of shrimp allergic subjects. One year later, the same group of investigators identified a sarcoplasmic-binding protein (Lit v 4) in *Litopenaeus vannamei* (28). Lit v 4 presents structural homology with Pen a 4 of *Penaeus aztecus* (29). Immunoblotting demonstrated IgE binding to a 20 kDa shrimp protein in 31 out of 52 (59.6%) sera of the shrimp allergic individuals. In addition, α-actinin, β-actin, fructose biphosphate aldolase, and ubiquitin have been identified as allergens in the *Solenocera melantho* (red shrimp) species (30), and other authors have identified the hemocyanin C subunit as an allergen in our shrimp-allergic patients (31). Hemocyanin is a hemolymph allergen with sequence homology of 62.5 - 100% with several crustacea hemocyanins (32). Paramyosin, an invertebrate-specific myofibrillar protein, is a thermo-labile 100 kDa allergen recently identified as an allergen in various shellfish (33). An amino acid sequence homology as high as 70% was recognized between disc abalone *Haliotis discus discus* and Mediterranean mussel paramyosins.
Currently, the role of the allergens different to tropomyosin in shrimp allergy has not yet been well defined. Asero et al. showed that Italian adult shrimp-allergic individuals react to a wide variety of allergens, that tropomyosin is the relevant allergen only in a minority of patients, and that a large proportion of subjects react to not-identified to date high molecular weight allergen proteins (34).

Current treatment for shrimp allergy

This section will not review the treatment of an acute allergic reaction, but the two currently existing etiological therapeutic approaches: desensitization and avoidance.

Desensitization

The natural course of shrimp allergy is to persist over time. Disappearance of reactivity to shrimp is rare. It is firmly established that Allergen Specific Immunotherapy (ASIT) is the only etiological treatment that can alter the natural course of allergic diseases, such as allergic rhino-conjunctivitis and asthma (35,36). The beneficial or harmful effect of HDM ASIT in shrimp allergy is still controversial. Usually, commercial HDM extracts used for SCIT contain quantified levels of group 1 and 2 allergens, but may also include low concentrations of other sensitizing allergens, such as tropomyosin (37). Pevec et al. studied 56 HDM-allergic patients treated with SCIT using HDM extract during 3 - 5.5 years. Specific gE (sIgE) to tropomyosin were found only in 5 patients, without clinical importance. Authors concluded that ASIT using HDM extracts does not induce clinically relevant sensitization to tropomyosin. Van Ree et al. studied 17 sera of HDM allergic patients receiving HDM immunotherapy (38). Serum samples were taken at the start of immunotherapy and 14-20 months later. At the beginning of immunotherapy, specific IgE for shrimp were positive in 3/17 subjects. After 14 to 20 months of immunotherapy, IgE responses for Der p 1 and Der p 2 were not increased. The 3 patients with initial positive specific IgE to shrimp were the only patients who had clinical symptoms after eating shrimps. So, it seems that immunotherapy for HDM does not increase the risk of reaction after ingestion of shrimp in previously non-sensitized patients. In a study with HDM allergic individuals non-sensitized to tropomyosin, Asero et al. observed that injection SIT with HDM extracts did not seem to induce de novo tropomyosin sensitization in mite-allergic patients after three years of ASIT

(39). Pajno et al. observed worsening of snail allergic reactions after several months of ASIT in children with previous combined mite-snail allergy (40).
Therefore, HDM immunotherapy has been reported to induce either shrimp allergy in non-allergic patients, shrimp tolerance in shrimp-allergic patients or no effect on shrimp sensitization. The contradictory results observed could be due to the different qualities of the HDM extracts used for immunotherapy, especially their content of minor allergens such as Der p 10. Possibly, ASIT with an extract containing quantified tropomyosin could induce desensitization in shellfish and HDM allergic patients. Actually, Cortellini et al. published a case report of a 15-year-old male diagnosed as having shrimp allergy and HDM allergy, who tolerated ingestion of shrimps after 12 months of sublingual immunotherapy (SLIT) for HDM (41). Authors hypothesized that the improvement in respiratory symptoms for HDM and in the food challenge for shrimps during mite immunotherapy with a known and high dosage of tropomyosin, may be dose dependent.

Avoidance measures

Food intake avoidance is the basic measure in managing food allergies. Generally, patients with food allergy to shrimp do not experience respiratory symptoms on inhalation of steam from cooking shrimp. But in some hypersensitive patients, airborne exposure might cause severe respiratory symptoms (15). Food avoidance seems theoretically easier than aeroallergens avoidance. Because of cross-reactivity, avoidance of all crustaceans is generally advised. Shrimp allergic patients are usually healthy, unless they suffer an acute episode by accidental ingestion of shellfish. So, these patients have no awareness of the disease and of the consequences of this problem. Therefore, educational programs are a keystone of the management of food allergy. The patient and her/his family should receive full information about the disease, its consequences, the recognition of the symptoms of an allergic reaction, and training about self-administration of drugs such as epinephrine. Re-education about diet intake of the patients and their families is also important. Patients should be alerted to possible hidden allergen exposure, particularly in restaurants where cooking equipment or serving utensils may be used for different foods. In addition, in many cases, the assessment by a specialist in nutrition would be adequate.

Future therapies for shrimp allergy

Production of hypoallergenic shrimp

Hypoallergenic foods are those in which the allergenic capacity has been reduced by altering the structure of allergenic epitopes, reducing the risk of reaction when consumed by allergic patients. You can obtain hypoallergenic foods by physical, chemical and genetic modification procedures (42).

Physical treatments
Usui et al. investigated the effects of tropomyosin concentrations from cooked or raw shrimp (43). The risk of shrimp allergic reaction was not reduced significantly by different methods of preparing cooked shrimp.

Chemical procedures: enzymatic hydrolysis
Through enzymatic hydrolysis of shrimp, hypoallergenic products could be obtained which, while retaining some residual allergenicity, are tolerated by most shrimp allergic patients. Wang used proteases such as trypsin, papain and bromelain individually, to hydrolyze South American shrimp proteins (44). The results showed that hydrolysates from South American shrimp proteins digested by each of the three proteases, better with papain, could result in an attenuation of general allergic reactions.

Modified immunotherapy

Vaccination with DNA plasmids
Wai et al. constructed two hypoallergens of the shrimp tropomyosin Met e 1: MEM49 and MED171, and expressed them in plasmid pCI-Neo (45). Authors concluded that hypoallergen-based DNA vaccines could effectively protect against tropomyosin sensitization in mice via the establishment of Th1-oriented responses, recruitment of regulatory T cells, and induction of blocking IgG antibodies.

T cell epitope immunotherapy
Peptide-based immunotherapy (PIT) has been found to successfully treat allergic patients, and there are many opportunities for the application of PIT in this category (46). Wai et al. identified the immunodominant T cell epitopes of tropomyosin of *Metapenaeus ensis* (Met a 1), and evaluated their therapeutic effect in a Balb/c mouse model of Met e 1 hypersensitivity (47). Mice treated with the T cell epitope peptide mixture demonstrated an amelioration of systemic allergic symptoms, and a significant reduction in Th2-associated antibody and cytokine responses. More investigators are identifying CD4 T cell shrimp tropomyosin-derived epitopes for the design of PIT of shrimp-allergic patients (48).

Probiotics

The beneficial effect of probiotics has been demonstrated in the treatment of allergic diseases. Probiotics interact with the host immune system and may provide preventive and therapeutic effects on allergic diseases (49). Schiavi et al. investigated the therapeutic potential of VSL#3 probiotic mixture on specific immune responses and anaphylactic reaction induced in mice

Table I *- Future therapies for shrimp allergy.*

Production of hypoallergenic shrimp	physical treatments chemical procedures
Modified immunotherapy	vaccination with DNA plasmids T cell epitope immunotherapy
Probiotics	
Chinese herbal formulations	

by shrimp tropomyosin (50). Oral therapeutic administration of VSL#3 to tropomyosin sensitized mice significantly reduces symptom score and histamine release in faeces after allergen challenge. Measurements of IL-4, IL-5 and IL-13 were significantly reduced, whereas FOXP3 and IL-27 mRNA expression, IL-10, TGF-β and IFN-γ showed higher levels.

Chinese Herbal Formulations (CHF)

Plants have been used for medicinal purposes for thousands of years. Traditional Chinese medicine, which has been practiced for centuries in Asia, has awakened the interest of Western countries as an alternative or complementary therapy for some diseases, including allergic diseases such as food allergy. Li et al. tested the effect of a mixture of 11 herbs in an experimental model of mice with allergy to peanut. In this study protection was observed in the mouse, avoiding the appearance of anaphylactic reaction in the test of provocation with peanut (51). In addition, after two weeks of treatment levels of specific IgE were significantly reduced, and remained low for at least 4 weeks after discontinuation of treatment. Also a reduction in the synthesis of cytokines Th2, IL-4, IL-5 and IL-13 was observed, without altered IFN-γ production. A major limitation of the current presentation of CHF is the inconveniently high daily dose. Therapeutic use of Chinese herbal formulations on food allergy is currently being investigated in several clinical trials with promising results in several food allergies including shellfish allergy (52).

In **Table I** we present the future therapies for shrimp allergy included in this review, summarized.

Conclusions

This article reviews as current as possible the future therapies for shrimp allergy. At the moment, there is a therapeutic need for this food allergy. The attempts in exploring new options, as alternative therapy, will lead to new treatments for this and for other food allergies. Our food allergic patients have been expecting for it for too many years.

References

1. Abowei JFN, Ezekiel E. Potentials and uses of fish products and other aquatic animals. Scientia Agriculturae 2013; 3(3):70-81.
2. Futamura M, Kato K, Hirose I, Morishita M, Ito K, Kakami M, et al. Vitamin D-deficient rickets in a young child with fish allergy. Arerugi 2003; 52(6):530-3.
3. Barreto-Chang OL, Pearson D, Shepard WE, Longhurst CA, Greene A. Vitamin D--deficient rickets in a child with cow's milk allergy. Nutr Clin Pract 2010; 25(4):394-8.
4. Malo JL, Chretien P, McCants M, Lehrer S. Detection of snow-crab antigens by air sampling of a snow-crab production plant. Clin Exp Allergy 1997; 27(1):75-8.
5. Malo JL, Cartier A. Occupational reactions in the seafood industry. Clin Rev Allergy 1993; 11(2):223-40.
6. Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. Lancet 1994; 343(8906):1127-30.
7. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, et al. Prevalence of adverse reactions to food in Germany - a population study. Allergy 2004; 59(3):338-45.
8. Tejedor-Alonso MA, Moro-Moro M, Mugica-Garcia MV. Epidemiology of anaphylaxis: contributions from the last 10 years. J Investig Allergol Clin Immunol 2015; 25(3):163-75; quiz follow 74-5.
9. Asero R, Antonicelli L, Arena A, Bommarito L, Caruso B, Colombo G, et al. Causes of food-induced anaphylaxis in Italian adults: a multi-centre study. Int Arch Allergy Immunol 2009; 150(3):271-7.
10. Maulitz RM, Pratt DS, Schocket AL. Exercise-induced anaphylactic reaction to shellfish. J Allergy Clin Immunol 1979; 63(6):433-4.
11. Harada S, Horikawa T, Ashida M, Kamo T, Nishioka E, Ichihashi M. Aspirin enhances the induction of type I allergic symptoms when combined with food and exercise in patients with food-dependent exercise-induced anaphylaxis. Br J Dermatol 2001; 145(2):336-9.
12. Pascual CY, Crespo JF, Perez PG, Esteban MM. Food allergy and intolerance in children and adolescents, an update. Eur J Clin Nutr 2000; 54 Suppl 1:S75-8.
13. Bohlke K, Davis RL, DeStefano F, Marcy SM, Braun MM, Thompson RS, et al. Epidemiology of anaphylaxis among children and adolescents enrolled in a health maintenance organization. J Allergy Clin Immunol 2004; 113(3):536-42.
14. Lehrer SB, Ibanez MD, McCants ML, Daul CB, Morgan JE. Characterization of water-soluble shrimp allergens released during boiling. J Allergy Clin Immunol 1990; 85(6):1005-13.
15. Asero R, Mistrello G, Roncarolo D, Amato S. A case of allergy to airborne, heat-labile shrimp allergens. J Allergy Clin Immunol 2002; 109(2):371-2.

16. Naqpal S, Rajappa L, Metcalfe DD, Rao PV. Isolation and characterization of heat-stable allergens from shrimp (Penaeus indicus). J Allergy Clin Immunol 1989; 83(1):26-36.
17. Reese G, Ayuso R, Lehrer SB. Tropomyosin: an invertebrate pan-allergen. Int Arch Allergy Immunol 1999; 119(4):247-58.
18. Leung PS, Chow WK, Duffey S, Kwan HS, Gershwin ME, Chu KH. IgE reactivity against a cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen. J Allergy Clin Immunol 1996; 98(5 Pt 1):954-61.
19. Leung PS, Chen YC, Gershwin ME, Wong SH, Kwan HS, Chu KH. Identification and molecular characterization of Charybdis feriatus tropomyosin, the major crab allergen. J Allergy Clin Immunol 1998; 102(5):847-52.
20. Leung PS, Chen YC, Mykles DL, Chow WK, Li CP, Chu KH. Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen. Mol Mar Biol Biotechnol. 1998;7(1):12-20.
21. Castillo R, Delgado J, Quiralte J, Blanco C, Carrillo T. Food hypersensitivity among adult patients: epidemiological and clinical aspects. Allergol Immunopathol (Madr) 1996; 24(3):93-7.
22. Yi FC, Cheong N, Shek LP, Wang DY, Chua KY, Lee BW. Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in Blomia tropicalis and Dermatophagoides pteronyssinus. Clin Exp Allergy 2002; 32(8):1203-10.
23. Wong L, Huang CH, Lee BW. Shellfish and house dust mite allergies: is the link tropomyosin? Allergy Asthma Immunol Res 2016; 8(2):101-6.
24. Jarrett P, Scragg R. A short history of phototherapy, vitamin D and skin disease. Photochem Photobiol Sci 2016.
25. Yu CJ, Lin YF, Chiang BL, Chow LP. Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. J Immunol 2003; 170(1):445-53.
26. Uda K, Fujimoto N, Akiyama Y, Mizuta K, Tanaka K, Ellington WR, et al. Evolution of the arginine kinase gene family. Comp Biochem Physiol Part D Genomics Proteomics 2006; 1(2):209-18.
27. Ayuso R, Grishina G, Bardina L, Carrillo T, Blanco C, Ibanez MD, et al. Myosin light chain is a novel shrimp allergen, Lit v 3. J Allergy Clin Immunol 2008; 122(4):795-802.
28. Ayuso R, Grishina G, Ibanez MD, Blanco C, Carrillo T, Bencharitiwong R, et al. Sarcoplasmic calcium-binding protein is an EF-hand-type protein identified as a new shrimp allergen. J Allergy Clin Immunol 2009; 124(1):114-20.
29. Ortea I, Canas B, Calo-Mata P, Barros-Velazquez J, Gallardo JM. Identification of commercial prawn and shrimp species of food interest by native isoelectric focusing. Food Chem 2010; 121(2):569-74.
30. Gamez C, Zafra M, Boquete M, Sanz V, Mazzeo C, Ibanez MD, et al. New shrimp IgE-binding proteins involved in mite-seafood cross-reactivity. Mol Nutr Food Res 2014; 58(9):1915-25.
31. Giuffrida MG, Villalta D, Mistrello G, Amato S, Asero R. Shrimp allergy beyond Tropomyosin in Italy: clinical relevance of Arginine Kinase, Sarcoplasmic calcium binding protein and Hemocyanin. Eur Ann Allergy Clin Immunol 2014; 46(5):172-7.
32. Piboonpocanun S, Jirapongsananuruk O, Tipayanon T, Boonchoo S, Goodman RE. Identification of hemocyanin as a novel non-cross-reactive allergen from the giant freshwater shrimp Macrobrachium rosenbergii. Mol Nutr Food Res 2011; 55(10):1492-8.
33. Suzuki M, Shimizu K, Kobayashi Y, Ishizaki S, Shiomi K. Paramyosin from the Disc Abalone Haliotis Discus Discus. Journal of Food Biochemistry 2014; 38(4):444-51.
34. Asero R, Mistrello G, Amato S, Ariano R, Colombo G, Conte ME, et al. Shrimp allergy in Italian adults: a multicenter study showing a high prevalence of sensitivity to novel high molecular weight allergens. Int Arch Allergy Immunol 2012; 157(1):3-10.
35. Viswanathan RK, Busse WW. Allergen immunotherapy in allergic respiratory diseases: from mechanisms to meta-analyses. Chest 2012; 141(5):1303-14.
36. Abramson MJ, Puy RM, Weiner JM. Injection allergen immunotherapy for asthma. Cochrane Database Syst Rev 2010; (8):CD001186.
37. Arlian LG, Morgan MS, Vyszenski-Moher DL, Sharra D. Cross-reactivity between storage and dust mites and between mites and shrimp. Exp Appl Acarol 2009; 47(2):159-72.
38. van Ree R, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Possible induction of food allergy during mite immunotherapy. Allergy 1996; 51(2):108-13.
39. Asero R. Lack of de novo sensitization to tropomyosin in a group of mite-allergic patients treated by house dust mite-specific immunotherapy. Int Arch Allergy Immunol 2005; 137(1):62-5.
40. Pajno GB, La Grutta S, Barberio G, Canonica GW, Passalacqua G. Harmful effect of immunotherapy in children with combined snail and mite allergy. J Allergy Clin Immunol 2002; 109(4):627-9.
41. Cortellini G, Spadolini I, Santucci A, Cova V, Conti C, Corvetta A, et al. Improvement of shrimp allergy after sublingual immunotherapy for house dust mites: a case report. Eur Ann Allergy Clin Immunol 2011; 43(5):162-4.
42. Martorell A, Martin Muñoz MF, Porcel S, Dalmau Serra J, Martin Esteban M. Prevencion y tratamiento de la alergia a los alimentos. In: Pelaez A, Davila I, editors. Tratado de Alergologia. Ergon Publisher, Madrid, 2005: 982.
43. Usui M, Harada A, Yasumoto S, Sugiura Y, Nishidai A, Ikarashi M, et al. Relationship between the risk for a shrimp allergy and freshness or cooking. Biosci Biotechnol Biochem 2015; 79(10):1698-701.
44. LJ Wang. Elimination of Allergens in South American Shrimp by Protease Hydrolysis. Food Science 2010; 31(17):263-6.
45. Wai CYY, Leung NYH, Ho MHK, Gershwin LJ, Shu SA, Leung PSC, et al. Immunization with Hypoallergens of Shrimp Allergen Tropomyosin Inhibits Shrimp Tropomyosin Specific IgE Reactivity. PLOS ONE 2014; 9(11):e111649.
46. El-Qutob D, Reche P, Subiza JL, Fernandez-Caldas E. Peptide-based allergen specific immunotherapy for the treatment of allergic disorders. Recent Pat Inflamm Allergy Drug Discov 2015; 9(1):16-22.
47. Wai CY, Leung NY, Leung PS, Chu KH. T cell epitope immunotherapy ameliorates allergic responses in a murine model of shrimp allergy. Clin Exp Allergy 2016; 46(3):491-503.
48. Ravkov EV, Pavlov IY, Martins TB, Gleich GJ, Wagner LA, Hill HR, et al. Identification and validation of shrimp-tropomyosin specific CD4 T cell epitopes. Hum Immunol 2013; 74(12):1542-9.
49. Yan F, Polk DB. Probiotics and immune health. Curr Opin Gastroenterol 2011; 27(6):496-501.
50. Schiavi E, Barletta B, Butteroni C, Corinti S, Boirivant M, Di Felice G. Oral therapeutic administration of a probiotic mixture suppresses established Th2 responses and systemic anaphylaxis in a murine model of food allergy. Allergy 2011; 66(4):499-508.
51. Li XM, Zhang TF, Huang CK, Srivastava K, Teper AA, Zhang L, et al. Food Allergy Herbal Formula-1 (FAHF-1) blocks peanut-induced anaphylaxis in a murine model. J Allergy Clin Immunol 2001; 108(4):639-46.
52. Wang J, Jones SM, Pongracic JA, Song Y, Yang N, Sicherer SH, et al. Safety, clinical, and immunologic efficacy of a Chinese herbal medicine (Food Allergy Herbal Formula-2) for food allergy. J Allergy Clin Immunol 2015; 136(4):962-70 e1.

ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 49, N 6, 257-262, 2017

M. Dogru[1], L.P. Seren[1]

Serum 25-hydroxyvitamin D levels in children with recurrent wheezing and relation to the phenotypes and frequency of wheezing

[1] Zeynep Kamil Woman and Children' Diseases Training and Research Hospital, Department of Pediatrics, Istanbul, Turkey

Key Words
wheezing; vitamin D; child; episodes; phenotype

Corresponding author
Mahmut Doğru
Zeynep Kamil Woman and Children' Diseases Training and Research Hospital
Department of Pediatrics, Burhanettin Ustunel Cad. 10
Uskudar, Istanbul, Turkey
Phone: +90 505 270 35 14
Fax: +90 216 391 06 99
E-mail: mdmahmut@yahoo.com

Doi
10.23822/EurAnnACI.1764-1489.14

Summary

Background. *Recurrent wheezing may be related to various reasons. There is a lack of knowledge about the effect of vitamin D status in the children with recurrent wheezing. The aim of this study is to compare the level of vitamin D between recurrent wheezing children and healthy controls, and to investigate the relationship between vitamin D status and the clinical parameters of recurrent wheezing in preschool children.* ***Methods.*** *One hundred-ten children followed up in our hospital with recurrent wheezing were included in the study. The control group included fifty children without wheezing episodes. The serum 25-hydroxyvitamin D (25OHD) level was measured. The patients with recurrent wheezing were grouped according to their vitamin D status as "deficient group" and "non-deficient group (Vitamin D level is insufficient and normal)". We investigated the relationship between vitamin D status and the clinical and laboratory parameters of children with recurrent wheezing.* ***Results.*** *Mean 25OHD level was 21.66 ± 8.13 ng/mL (5.6-53) in the study group and 25.36 ± 10.17 ng/mL (6-59) in the control group. The difference was statistically significant (p = 0.015). When the patients with recurrent wheezing were compared according to their vitamin D status, number of hospitalizations, number of positive sensitivity, percentage of eosinophil, serum IgE levels, Asthma Predictive Index positivity and wheezing phenotypes were not found to be different between groups. However, the duration of wheezing, the number of wheezing episodes and systemic glucocorticoid need in the previous year, and the total number of wheezing episodes were significantly higher in the deficient group (p < 0.05). The serum 25OHD level was negatively correlated with the duration of wheezing (r: -0.238; p: 0.012), total number of wheezing episodes (r: -0.436; p: 0.001), number of wheezing episodes in the previous year (r: -0.395; p: 0.001), and systemic glucocorticoid need in the previous year (r: -0.324; p: 0.001).* ***Conclusions.*** *Mean 25OHD levels were lower in patients with recurrent wheezing than in healthy controls. The duration of illness and number of wheezing episodes were correlated with vitamin D levels. An evaluation of the serum levels of vitamin D and supplementation if needed should be recommended in patients with recurrent wheezing, especially in those with long-term and frequent wheezing episodes.*

Introduction

Wheezing is one of the most common respiratory symptoms in the childhood. Allergic sensitization in early life, infection with rhinovirus, or colonization with any of a number of bacteria have been associated with increased risk of persistent wheeze. Acute respiratory infection is the main cause of recurrent wheezing in children. Respiratory viruses play a key role in the development and exacerbation of obstructive respiratory diseases in children (1,2). Recent studies showed that vitamin D acts as a hormone and takes a role in the function of many organs in addition to bone. Vitamin D deficiency predisposes to infections by affecting the production of antimicrobial peptides such as Cathelicidin. The antimicrobial activity against airway pathogens is increased with vitamin D treatment because of antimicrobial cationic peptides, defensin-beta-2 and 4 regulated by vitamin D. 1,25-dihydroxyvitamin D is the active form of vitamin D. 1,25-dihydroxyvitamin D decreased proliferation of Th1 and Th2 cells, and production of interferon (IFN)-ɣ, interleukin (IL)-2 and IL-5. But IL-4 production is increased by 1,25-dihydroxyvitamin D (3,4). Previous studies have shown that low serum 25-hydroxyvitamin D (25OHD) levels are associated with increased risk of respiratory tract infections in children and adults (5-7). There are some studies supporting the relationship between vitamin D deficiency and recurrent wheezing in childhood (7-11) as well as studies showing no relationship (12) or negative relationship (13). We aimed to compare the level of vitamin D between children with recurrent wheezing and healthy controls, and to investigate the relationship between vitamin D status and the clinical parameters of recurrent wheezing in preschool children.

Materials and methods

A total of 160 children (64 girls, 96 boys; aged 0.75-5 years) enrolled in the study. The study was approved by the local ethics committee of the same institute and adhered to the principles of Helsinki Declaration. A consent was obtained from all subjects and/ or their parents.

Study Population

One hundred-ten children with recurrent wheezing who started before 3 years and had more than 3 wheezing episodes were included in the study. The control group consisted of fifty children without wheezing episodes or any chronic disease. Children were evaluated in September 2012-February 2013 and September 2013-February 2014, in order to exclude the seasonal differences. The patients' age, gender, exposure to smoke, personal/ familial atopy and wheezing history were recorded. Presence of atopy in the family was considered as positive if a first-degree relative (mother, father, sibling) had allergic disease. Milk consumption of cases was not evaluated, because milk is not fortified with vitamin D in our country. The percentage of eosinophils, immunoglobulin (Ig) E levels and serum 25OHD levels were measured. RW patients were classified according to the number of wheezing episodes in the previous year as having had up to 3 episodes / year (n = 61) or > 3 episodes / year (n = 49).

Excluding Criteria

Patients receiving multivitamin support or any systemic glucocorticoid therapy, patients with obesity (body mass index > 95.p), patients with the clinical findings of rickets (o-bain, x-bain etc.), with any (clinical or laboratory) history of congenital heart disease, chronic lung disease (such as cystic fibrosis, bronchiectasis), tuberculosis, bronchopulmonary dysplasia, immunodeficiency, neurologic or metabolic diseases were excluded.

The phenotyping of wheezing

The Asthma Predictive Index (API) is an index used to evaluate the risk of developing asthma in the future with children who had recurrent wheeze. The major criteria are physician-diagnosed asthma in parents and atopic dermatitis in the child. The minor criteria are eosinophilia (> 4%), physician diagnosed allergic rhinitis and wheezing without upper respiratory tract infection in the child. It was considered positive when at least one major or two minor criteria were present (14).
In another phenotyping of wheezing, children were classified as having either episodic wheezing (EW) (wheezing only during colds and remaining asymptomatic between episodes) or multiple-trigger wheezing (MTW) (wheezing during colds but symptomatic between episodes, with wheezing activated by factors such as house dust, tobacco smoke, exercise, crying, laughter, or odor) (15).

Skin Prick Tests

Skin prick tests were applied on the anterior forearm. Patients were considered eligible for the skin test if they had not received antihistamines for at least one week. Skin prick tests for common aeroallergens (*Dermatophagoides pteronyssinus, Dermatophagoides farinea*, mixture of grass pollens, a mixture of grain pollens (oats, wheat, barley, corn), a mixture of tree pollens, weed-mix pollens, *Alternaria alternaria*, cockroaches (*Blatella germanica*), cat dander and dog dander (Stallergenes SA, 92160 Antony, France) were performed by using Stallerpoint® (Stallergenes SA, 92160 Antony, France). Histamine (10 mg/ml) and physiological saline were used as positive and negative references, respectively. Skin reactions were evaluated at 20th minute of the application. A positive reaction was characterized as 3 mm

or greater than that of the negative control. Atopy was classified as at least 1 positive response to these allergens with no response to negative reference solution and a response to positive reference (histamine).

Vitamin D Levels

Vitamin D levels were measured 2 weeks after any infection. Peripheral venous blood samples were obtained from all children and serum 25OHD levels were measured by using liquid chromatography-tandem mass spectrometry (LC-MS-MS) method in Waters Quattro Premier XE™ (Waters Corp. Milford, USA). Serum 25OHD levels ≤ 20 ng/mL were considered as vitamin D deficiency, while levels between 20 and 29 ng/mL as vitamin D insufficiency, 30-80 ng/mL as optimal vitamin D level and ≥ 80 ng/mL as potential vitamin D toxicity (16). The patients with recurrent wheezing were grouped according to their vitamin D status as "deficient group" and "non-deficient group (Vitamin D levels insufficient and normal)".

Statistical Analyses

Data was analyzed by using the Statistical Package for Social Sciences (SPSS) for Windows (SPSS 15.0 Chicago, USA) program. Values for continuous variables were given as either mean ± standard deviation or as median (interquartile range), based on the normality of distribution. Student t test was used for the comparison of normally distributed variables. Mann-Whitney U test was used for non-normally distributed variables. Chi-Square test was used to compare categorical variables. Pearson's correlation test was used for the correlation analyses of continuous variables. $p < 0.05$ was considered as significant.

Results

A total of 160 children were enrolled in this study. The study group consisted of 110 children (mean age: 2.97 ± 1.26 years; 69 boys and 41 girls) and control group were 50 children without wheezing episodes (mean age: 2.89 ± 1.27 years; 27 boys and 23 girls). There were no statistically significant differences in regards to age and gender between patients and control groups. Mean 25OHD level was 21.66 ± 8.13 ng/mL (5.6-53) in the study group and 25.36 ± 10.17 ng/mL (6-59) in the control group. The difference was statistically significant ($p = 0.015$). Vitamin D status was determined as deficient in 50 (45.5%) patients, and non-deficient (insufficiency or normal) in 60 (54.5%) of the study group, while deficient in 14 (28%) and non-deficient in 36 (72%) of the control group. Vitamin D status was found to be different between groups ($p = 0.039$). When patients are grouped by API positivity; although mean 25OHD level in API negative group was lower than API positive group, this difference was not statistically significant (81 patients, 21.43 ± 8.78 ng/mL vs 29 patients, 22.30 ± 6.02 ng/mL; $p = 0.623$). Similarly, when the patients were grouped in terms of 25 OHD level according to the wheezing phenotypes (EW or MTW), there was no difference between groups (51 patients, 22.78 ± 9.08 ng/mL vs 59 patients, 20.69 ± 7.14 ng/mL; $p = 0.342$).

The patients with recurrent wheezing were grouped according to their vitamin D status as "deficient (Group-I)" and "non-deficient (Group-II)". No statistically significant difference was present between groups in terms of gender, age, familial atopy, and exposure to smoke ($p > 0.05$) (**table 1**).

The number of hospitalizations, number of positive sensitivity, percentage of eosinophils, and serum IgE levels were not found to be different between groups. However, the duration of wheezing, the number of wheezing episodes and systemic glucocorticoid need in the previous year and the total number of wheezing episodes were significantly different between groups ($p < 0.05$) (**table 1**).

When patients compared according to the number of wheezing episodes in the previous year (≤ 3 episodes/years and > 3 episodes / years), the mean vitamin D level was significantly lower in patients with > 3 episodes/years (49 patients, 19.36 ± 7.72 ng/mL vs 61 patients, 23.50 ± 8.04 ng/mL; $p = 0.007$).

Eighty patients (72.7%) of study group underwent to skin prick test. Mean 25OHD level was 20.86 ± 6.2 ng/mL in atopic children and 20.81 ± 8.53 ng/mL in non-atopic children. The difference was not statistically significant ($p = 0.977$). Vitamin D status was not found to be different between atopic children and non-atopic children ($p = 0.653$) (**table 1**).

The serum 25OHD level was negatively correlated with the duration of wheezing (r: -0.238; p: 0.012), total number of wheezing episodes (r: -0.436; p: 0.001), number of wheezing episodes (r: -0.395; p: 0.001) and systemic glucocorticoid need in the previous year (r: -0.324; p: 0.001).

Discussion

This study showed that mean 25OHD levels of children with recurrent wheezing is lower than healthy control group. This result is similar to the results of previous studies (7-11). The study of Bener et al (7) compared vitamin D status of allergic and healthy children and found that the frequency of severe vitamin D deficiency was significantly higher in children with wheezing (23.4%) than in healthy children (10.5%). They indicated that vitamin D deficiency was a significant risk factor for wheezing (relative risk = 1.29; $p = 0.05$). Similar results were found in the other studies (8-11). However, another study (12) compared vitamin D levels of 30 children with recurrent wheezing and 45 healthy children, and significant difference between groups were not found. The reason for the difference of the results of this study may be the small number of cases and age of the patients.

Table 1 *- Comparison of socio-demographic, clinical and laboratory findings features of the vitamin D deficient (Group-I), vitamin D non-deficient (Group-II) patients' groups.*

	Group I n: 50	**Group II n: 60**	**p**
Gender (Male/Female)	32/18	37/23	0.845[1]
Age (years)[‡]	3.1 ± 1.8	2.9 ± 1.3	0.303[2]
Familial atopy n (%)	15 (30)	24 (40)	0.320[1]
Exposure to smoke n (%)	26 (52)	33 (55)	0.848[1]
Duration of disease (Month)[*]	24 (15.5-30)	18 (12-25)	0.037[3]
Total numbers of wheezing episodes[*]	6 (5-10)	4 (3-5)	0.001[3]
Numbers of hospitalizations[*]	0 (0-1)	0 (0-0)	0.100[3]
Systemic glucocorticoid need in the previous year[*]	1 (0-2)	0 (0-1)	0.001[3]
Numbers of wheezing episodes in the previous year[*]	3 (2-5)	3 (2-3.5)	0.011[3]
The percentage of eosinophils (%)[*]	2 (1.4-3.5)	2.6 (1.3-5.2)	0.052[3]
Immunoglobulin E (IU/ml)[*]	70 (29-352)	56 (32-156)	0.651[3]
API positivity n (%)	11 (22)	18 (30)	0.390[1]
Phenotyping of wheezing n (%) Episodic wheezing Multiple-triggered wheezing	 24 (48) 26 (52)	 27 (45) 33 (55)	 0.848[1]
Sensitivity in skin prick test (atopy) n (%)	19 (48.8)	17 (41.5)	0.653[1]
The number of positive sensitivity[*]	0 (0-2)	0 (0-2)	0.528[3]

[*]Data are presented as either mean ± Standard deviation or as median (interquartile range) according to the distribution.

[1]chi-square test [2]Student t test [3]Mann-Whitney U test

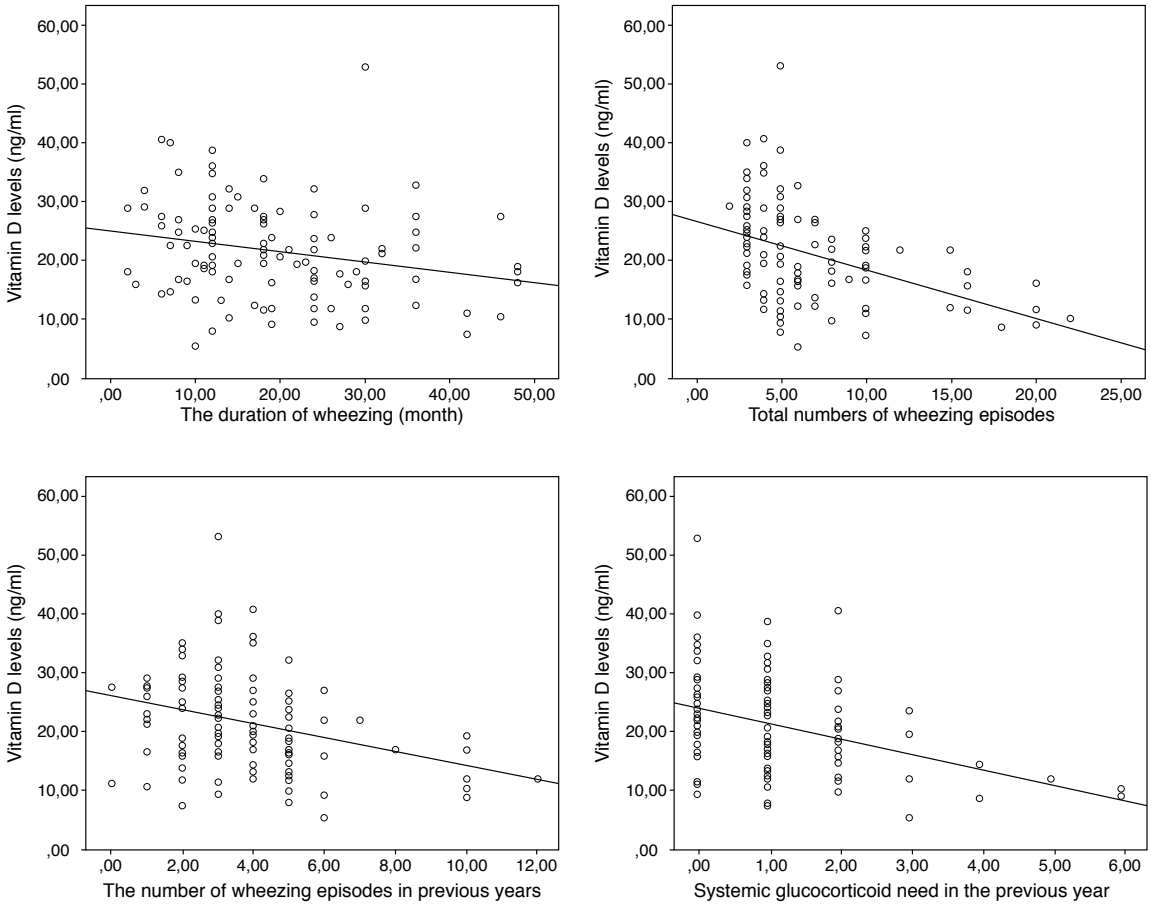
In another study, Pereira et al (13) assessed children with recurrent wheezing (RW) (n: 255) and occasional wheezing (OW) (n: 115). They observed significantly higher serum vitamin D levels in RW children than in OW children. This result is incompatible with our study. This difference may be related to the choice of patient. This study did not specify whether or not there were obese patients. In addition, the season of vitamin D measurement is uncertain in the study.

Vitamin D deficiency is an important health problem in both developed and developing countries. In this study 44.5% of children with recurrent wheezing was found to be vitamin D deficient while 28% of control group was vitamin D deficient. It was shown that vitamin D deficiency is a common problem among children in our country (17). The high frequency of vitamin D deficiency in our country may be related to inadequate vitamin D supplementation during infancy, low levels of vitamin D in pregnancy, scarcity of vitamin D fortified food products, and frequent respiratory tract infections.

The results of studies investigating vitamin D levels according to API and phenotypes of wheezing in children with RW are inconsistent (9,11). In a cross sectional study, the investigators reported a correlation of low levels of vitamin D in patients with RW, API positive and multi-trigger temporal pattern of wheeze compared with non-recurrent wheezing, API negative and episodic temporal pattern of wheeze. In a recent study in Turkey, Ozdemir et al (11) found that the API negative group had the lowest mean serum 25 (OH)D level, followed by API positive group compared to healthy group ($p < 0.05$). These differences in studies may depend on such factors as patient selection, age, time, method of measurement.

Another remarkable result of present study is the duration of wheezing, the high total number of wheezing episodes, number of wheezing episodes and systemic glucocorticoid need in the previous year among the vitamin D deficient wheezy children comparing to vitamin D non-deficient wheezy children. In addition, we found a negative correlation between these parameters and vitamin D levels. Uysalol et al (9) declared a negative correlation between vitamin D levels and the number of wheezing episodes, hospitalization and admission to emergency department in the previous year (r: -0.394 p = 0.010, r= -0.406 p

Figure 1 *- Distribution of the duration of wheezing, total numbers of wheezing episodes, numbers of wheezing episodes and systemic glucocorticoid need in the previous year with regard to vitamin D levels.*



< 0.001, r = 0.307 p = 0.008, respectively). Systemic steroid requiring episodes were not evaluated in this study, while Beigelman et al (18) reported an increased number of steroid requiring episodes in children with decreased vitamin D level. Increased frequency and severity of wheezing episodes in vitamin D deficient might be due to several reasons. Firstly, as mentioned above, increased frequency of respiratory infections is associated with vitamin D deficiency (5-7). Vitamin D deficiency has also been seen to correlate with an increased risk of respiratory viral co-infections, especially Respiratory syncytial virus and Rhinovirus infections. These viruses are considered the main triggers of childhood wheezing (19). Secondly, vitamin D has complex immunomodulatory properties for both innate and adaptive immune system functions as well as in calcium / phosphorus homeostasis and bone health. Vitamin D is implicated in fetal lung growth and maturation (20). There is also evidence of a role of vitamin D in maintaining lung structure and pulmonary function (21). Decreased lung maturation due to prematurity is a risk factor for recurrent wheezing. Because of vitamin D deficiency making deterioration in lung maturation and function, these patients are expected to have more frequent episodes wheezing. Third reason might be the suppressive effect of vitamin D on inflammation and airway hyper-responsiveness, by inhibiting synthesis and releasing inflammatory cytokines from bronchial smooth muscle cells. Vitamin D also promotes T-regulatory cell activity (22). Wheezing can occur more frequently due to the increased on inflammation and airway hyper-responsiveness caused by vitamin D deficiency.

Limited participant number was the negative aspect of our study. It was designed as a single-center study including 0.75-5 years old patients with RW. Vitamin D levels were measured in a single sample obtained in autumn or winter. More than one

sample could have been obtained and mean vitamin D levels throughout the year could have been measured in order to minimize the seasonal differences.

As a result, mean 25OHD levels were lower in patients with recurrent wheezing than healthy controls. The duration of illness and number of wheezing episodes were correlated with vitamin D levels. Evaluation of the serum levels of vitamin D and supplementation if needed should be recommended in patients with recurrent wheezing, especially in patients with long-term and frequent wheezing episodes.

References

1. Grad R, Morgan WJ. Long-term outcomes of early-onset wheeze and asthma. J Allergy Clin Immunol. 2012;130(2):299-307.
2. Takeyama A, Hashimoto K, Sato M, Sato T, Tomita Y, Maeda R, Ito M, Katayose M, Kawasaki Y, Hosoya M. Clinical and epidemiologic factors related to subsequent wheezing after virus-induced lower respiratory tract infections in hospitalized pediatric patients younger than 3 years. Eur J Pediatr. 2014;173(7):959-66.
3. Yim S, Dhawan P, Ragunath C, Christakos S, Diamond G. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D3. J Cyst Fibros. 2007;6(6):403-10.
4. Dimeloe S, Nanzer A, Ryanna K, Hawrylowicz C. Regulatory T cells, inflammation and the allergic response-The role of glucocorticoids and vitamin D. J Steroid Biochem Mol Biol. 2010;120:86-95.
5. Ginde AA, Mansbach JM, Camargo CA Jr. Association between serum 25- hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med. 2009;169:384-90.
6. Laaksi I, Ruohola JP, Tuohimaa P, Auvinen A, Haataja R, Pihlajamaki H, Ylikomi T. An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. Am J Clin Nutr. 2007;86:714-7.
7. Bener A, Ehlayel MS, Bener HZ, Hamid Q. The impact of Vitamin D deficiency on asthma, allergic rhinitis and wheezing in children: An emerging public health problem. J Family Community Med. 2014;21(3):154-61.
8. Ture M, Zeyrek CD, Koçyigit A. Serum vitamin D, folic acid and B12 levels in wheezy children. Asthma Allergy Immunol. 2013;11:169-77.
9. Uysalol M, Uysalol EP, Yilmaz Y, Parlakgul G, Ozden TA, Ertem HV, Omer B, Uzel N. Serum level of vitamin D and trace elements in children with recurrent wheezing: a cross-sectional study. BMC Pediatr. 2014;14(1):270.
10. Demirel S, Guner SN, Celiksoy MH, Sancak R. Is vitamin D insufficiency to blame for recurrent wheezing? Int Forum Allergy Rhinol. 2014;4(12):980-5.
11. Ozdemir A, Dogruel D, Yilmaz O. Vitamin D Status in Infants with Two Different Wheezing Phenotypes. Indian J Pediatr. 2016;83(12-3):1386-91.
12. Özaydın E, Bütün MF, Cakır BC, Köse G. The association between vitamin D status and recurrent wheezing. Indian J Pediatr. 2013;80(11):907-10.
13. Urrutia-Pereira M, Solé D. Is Vitamin D Deficiency a Marker of Severity of Wheezing in Children? A Cross-sectional Study. J Investig Allergol Clin Immunol. 2016;26(5):319-21.
14. Castro-Rodríguez JA, Holberg CJ, Wright AL, Martinez FD: A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Respir Crit Care Med. 2000, 162:1403-6.
15. Brand PL, Baraldi E, Bisgaard H, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J. 2008;32:1096-110.
16. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Duraz0-Arvizu RA, Gallagher JC, Gallo RL, Jones G, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. JCEM. 2011;96:53-8.
17. Andıran N, Çelik N, Akça H, Doğan G. Vitamin D deficiency in children and adolescents. J Clin Res Pediatr Endocrinol. 2012;4(1):25-9.
18. Beigelman A, Zeiger RS, Mauger D, Strunk RC, Jackson DJ, Martinez FD, Morgan JW, Covar R, Szefler SJ, Taussing LM, et al. Childhood Asthma Research and Education (CARE) Network of the National Heart, Lung, and Blood Institute. The association between vitamin D status and the rate of exacerbations requiring oral corticosteroids in preschool children with recurrent wheezing. J Allergy Clin Immunol. 2014;133(5):1489-92.
19. Jartti T, Ruuskanen O, Mansbach JM, Vuorinen T, Camargo CA Jr. Low serum 25-hydroxyvitamin D levels are associated with increased risk of viral coinfections in wheezing children. J Allergy Clin Immunol. 2010;126(5):1074-6.e1-4.
20. Sakurai R, Shin E, Fonseca S, Sakurai T, Litonjua AA, Weiss ST, Torday JS, Rehan VK. 1alpha,25(OH)2D3 and its 3-epimer promoter at lung alveolar epithelial-mesenchymal interactions and inhibit lipofibroblast apoptosis. Am J Physiol Lung Cell Mol Physiol. 2009;297(3):496-505.
21. Zosky GR, Berry LJ, Elliot JG, James AL, Gorman S, Hart PH. Vitamin D deficiency causes deficits in lung function and alters lung structure. Am J Respir Crit Care Med. 2011;183(10):1336-43.
22. MS Sandhu, TB Casale: The role of vitamin D in asthma. Ann Allergy Asthma Immunol. 2010;105(3):191-9.

D. Villalta[1], L. Cecchi[2], A. Farsi[2], F. Chiarini[2], P. Minale[3], S. Voltolini[3], E. Scala[4], O. Quercia[5], L. Muratore[6], V. Pravettoni[7], A.M. Calamari[8], G. Cortellini[9], R. Asero[10]

Galactose-α-1,3-galactose syndrome: an Italian survey

[1]Immunologia e Allergologia, Ospedale "S. Maria degli Angeli", Pordenone, Italy
[2]SOS Allergologia e Immunologia Clinica, USL Toscana Centro, Prato, Italy
[3]SOC di Allergologia, IRCCS S. Martino - IST, Genova, Italy
[4]Istituto Dermopatico dell'Immacolata - IRCCS, Roma, Italy
[5]Unità ad Alta Specializzazione di Allergologia, Ospedale di Faenza, Faenza, Italy
[6]Allergy and Clinical Immunology Service, Vito Fazio Hospital, Lecce, Italy
[7]UOC Clinical Allergy and Immunology, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy
[8]Ospedale Castelli, Pallanza, Italy
[9]UO di Medicina Interna e Reumatologia, Azienda Sanitaria della Romagna, Rimini, Italy
[10]Ambulatorio di Allergologia, Clinica S. Carlo, Paderno Dugnano, Milan, Italy

Key words

α-Gal syndrome; red meat allergy; cetuximab allergy; survey

Corresponding author
Danilo Villalta
Pordenone Hospital
Via Montereale 24, 33170 Pordenone, Italy
Phone: +39 0434 399647 281
Fax: +39 0434 399344
E-mail: danilo.villalta@aas5.sanita.fvg.it

Doi
10.23822/EurAnnACI.1764-1489.35

Summary

Background. *The term of α-Gal syndrome, which includes the delayed allergy to red meat and the allergic reactions following the administration of cetuximab, is associated to the presence of specific IgE to α-Gal. In Italy, only anecdotal cases were reported so far. The Association of Italian Allergists (AAITO) carried out a survey with the aim of evaluating presence, characteristics, clinical features, and distribution of the syndrome in Italy.* ***Methods.*** *A web structured questionnaire was made available on the website of AAIITO from July 2016 to January 2017. It included 31 multiple-choice questions concerning different items, including the site of physicians, the number of patients diagnosed as having cetuximab allergy and/or delayed red meat allergy, recall of tick bites, symptoms, time to reactions, elicitor foods, reactions with foods other than meat, and in-vivo and in-vitro tests used for the diagnosis.* ***Results.*** *Seventy-nine physicians completed the questionnaire. Nine cases of allergy to cetuximab and 40 cases of delayed red meat allergy were recorded across Italy. 22.5% of patients with cetuximab allergy and 62.5% of those with delayed red meat allergy recalled a tick bite. 75% of patients with delayed red meat allergy experienced symptoms after eating beef (butcher's cut in 72.5%). Urticaria was the most frequent clinical manifestation (65% of cases). In 60.6% of cases symptoms appeared 2 - 4 hours after meat ingestion, while in 7.9% symptoms appeared after > 4 hours. The most used diagnostic methods were the intradermal test for cetuximab allergy (88.9%) and the detection of IgE to α-Gal (55.5%) for red meat allergy. Most case reports came from Northern Italy.* ***Conclusion.*** *α-Gal syndrome is present in Italy and beef is the most frequent offending food. In most cases symptoms were not severe.*

Introduction

Galactose-α-1,3-galactose (α-Gal) is a carbohydrate epitope that is abundantly expressed on glycoproteins of mammalian origin, including non-primates, prosimians and New World monkeys (1,2) and is produced by the enzyme α-1,3-galactosyltranferase (α-1,3 GT). In contrast, α-Gal is not expressed in Old World monkeys, apes and humans, since the enzyme α-1,3 GT is non-functional, as result of an evolutionary event that occurred possibly 28 million years ago (3). As a consequence, species not expressing the α-Gal epitope produce large amounts of IgG antibodies to α-Gal, due to the constant antigenic stimulation exerted by bacteria present in the intestinal flora (4). It is estimated that approximately 1% of all IgG antibodies in human subjects are directed against the α-Gal epitope, representing the major immune barrier in xenotransplantation (5).
IgE antibodies against the α-Gal epitope were identified in a subset of patients treated with cetuximab, a chimeric mAb approved for use in patients with colorectal cancer and squamous cell carcinoma of the head and neck, who developed severe anaphylactic reactions upon the first administration, suggesting the presence of pre-existing IgE antibodies. Further investigations revealed that anaphylactic reactions were induced by pre-existing IgE antibodies against the α-Gal epitope on the Fab portion of cetuximab (6). In addition, IgE antibodies to α-Gal have resulted to be related also to delayed (from 3 to 6 hours) anaphylactic reactions following the ingestion of red meat (7,8). Intriguingly, both cetuximab and red meat allergies showed great variation depending on the geographic location, which led to hypothesize that the cross-reactivity could originate from locally occurring biting insect or other parasites (9). In the US, the development of IgE to α-Gal was eventually linked to bites from ecto-parasitic ticks, especially the Lone Star tick, *Amblyomma americanum* (10), whereas in Europe and Australia it has been associated to bites of *Ixodes* species (9). Hamsten and coworkers (11) showed that the α-Gal epitope is present in the gastrointestinal tract of *Ixodes ricinus,* which exposes the host to α-Gal during the bite. Two other papers from Brazil (12) and Japan (13) demonstrated the presence of α-Gal epitope in the saliva of other tick species, respectively *Ambyomma sculptum* and *Haemaphysalis longicornis*.
The term "α-Gal syndrome" was recently proposed to better describe this novel disease that occurs worldwide (14,15) and is clinically defined by the three facets of this allergy: (a) an IgE mediated food allergy with a typically delayed onset following the ingestion of mammalian meat and innards; (b) a drug allergy to cetuximab or gelatin-based colloids, both containing many α-Gal epitopes; and (c) allergic (generally local) reactions to tick bites (16).
Many cases of α-Gal syndrome have been described in Europe, particularly in Sweden, Germany and France (16,17), whereas in Italy only anecdotal cases have been reported so far (18), although a study carried out on a population living in pre-Alps area and largely exposed to bites of *Ixodes ricinus* showed a high prevalence of sensitization to α-Gal (19). In this study we report the results of a survey on α-Gal syndrome carried out among the members of the AAIITO (the Italian Association of Allergists and Immunologists working on the Territory) with the aim of evaluating the presence, clinical features, and distribution of α-Gal syndrome in Italy.

Methods

A web structured questionnaire was made available on the AAIITO website (www.aaiito.it) for six months, from July 2016 to January 2017. An invitation to participate to the survey was sent twice by e-mail to all the 500 members of the association. The 31 multiple-choice questions covered the following items: geographical origin of the physicians involved in the survey; number of patients visited per year; number of patients with cetuximab and/or delayed red meat allergy diagnosed so far; memory of a tick bite (if positive, where and number); symptoms features; in the case of red meat allergy: kind of meat involved, dish preparation, latency of reactions, reactions with foods other than meat; in vivo and in vitro diagnostic tests commonly used (20-21); availability of tests to detect sIgE to α-Gal.
In 44 centres, the diagnosis of α-Gal sensitization was carried out by means of ImmunoCap to a-Gal (Thermofisher diagnostics, Uppsala, Sweden).

Results

Seventy-nine physicians (79/500; 15.8%) from 20 of 21 Italian Regions (**Figure 1**) completed the questionnaire. They globally visit more than 150.000 patients per year (range 500 - > 2000 patients / centre).
Nine cases of allergy to the first injection of cetuximab were described in 5 centres (2 centres reported 3 cases) from 4 different regions (Toscana, Friuli Venezia Giulia, Lombardia and Lazio); specific features are shown in **Table I**. Only two patients recalled a previous tick bite. 4/9 (44.4%) experienced a severe reaction and the diagnosis was made by the intradermal test with cetuximab in the majority of patients (88.9%).
Forty cases of delayed red meat allergy were reported by 21 centres located in 11 different Italian Regions, with a higher prevalence in Northern Italy (**Figure 2**). The distribution of these cases in the 21 centres are shown in **Figure 3**, while their main features are reported in **Table II**. Twenty-five/40 (62.5% of patients) recalled a previous tick bite which, in the majority of cases, occurred in the hills (76%) or in the mountains (20%). 22/40 (55.3%) of patients experienced from two to four adverse reactions before the diagnosis, and 34.2% of pa-

Figure 1 *- Number of allergy centers distributed for the single Italian regions that answered the questionnaire.*

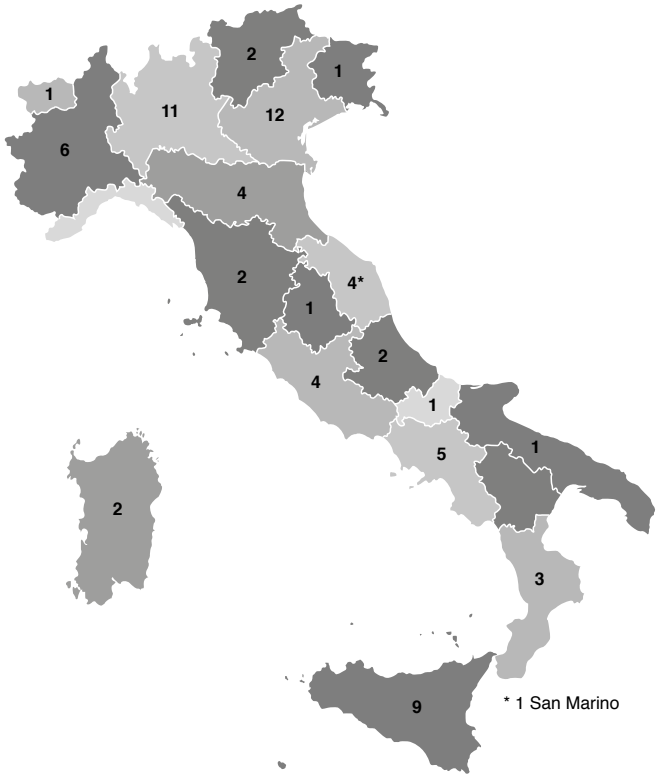


Table I *- Features of patients with cetuximab allergy.*

Question	N° (%)
1. Do the patient remember the tick bite	
yes	2/9 (22.2)
no	5/9 (55.6)
unknown	2/9 (22.2)
2. If yes, how many months before the adverse reaction?	
< 1	0/2 (0.0)
1-3	2/2 (100)
4-6	0/0 (0.0)
7-12	0/0 (0.0)
> 12	0/0 (0.0)
3. The patient was bitten	
in urban area	1/2 (50.0)
in rural area on the plain	1/2 (50.0)
in the hills	0/2 (0.0)
in the mountains	0.0 (0.0)
4. The adverse reaction was	
mild	2/9 (22.2)
moderate	3/9 (33.4)
severe	4/9 (44.4)
10. Methods used for the diagnosis	
SPT with cetuximab	6/9 (66.7)
Intradermal test with cetuximab	8/9 (88.9)
BAT	0/9 (0.0)
sIgE to α-Gal	3/9 (33.3)

tients more than four. 75% of the reactions were associated to the ingestion of beef, 17.5% of pork, and only few cases to the ingestion of sheep (5.0%) or game meat (2.5%). The majority of reactions (72.5%) were elicited by butcher's cut meat (i.e., muscle meat), whereas tripe (10%), sausages (5.0%), ham (2.5%), hamburger (2.5%) and liver / kidney (2.5) were the elicitors in the remaining cases. Nine out of 40 (22.5%) patients with delayed meat allergy reported adverse reactions also with other foods, in particular milk (4/9), gelatine containing sweet / toffee (4/9), and cakes (1/9), and one patient experienced an acute reaction during a gelatine plasma expander infusion. The most frequent clinical presentation was urticaria, and in 60.6% of patient the symptoms appeared between 2 and 4 hours after meat ingestion.

55% of the interviewed centres diagnosed α-Gal sensitization by in vitro means (specific IgE to α-Gal; Thermofisher diagnostics, Uppsala, Sweden); 55% by SPT with commercial beef and/or pork meat extracts, 42.5% by prick-prick with fresh meat, 27.5% by prick-prick with liver / kidney of pork or beef, 10% by SPT / intradermal test with cetuximab, and 5% by basophils activation test (BAT). α-Gal-specific IgE levels were very high in most cases (> 100 kUA/L in 8/22 cases) (**Figure 4**).

Finally, physicians were asked whether they felt that they previously visited other patients with possible α-Gal syndrome that remained undiagnosed, because at that time the syndrome was not yet known or because the diagnostic facilities were unavailable in their clinical setting. Nineteen physicians answered they met patients with symptoms possibly suggesting an α-Gal syndrome (17 cases of delayed red meat allergy; 2 cases of cetuximab allergy). In these cases, the diagnosis made was: idiopathic anaphylaxis (38.1%); adverse reaction to red meat / cetuximab of possible allergic type (19.0%); not allergic adverse reaction to red meat / cetuximab (9.5%); other diagnoses (33.3%).

Figure 2 *- Distribution of the cases of delayed red meat allergy in Italy.*

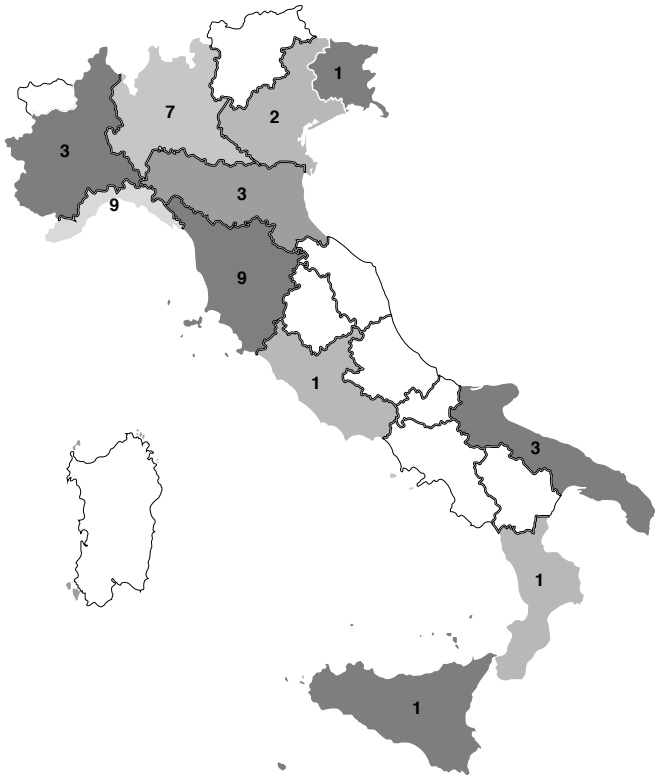
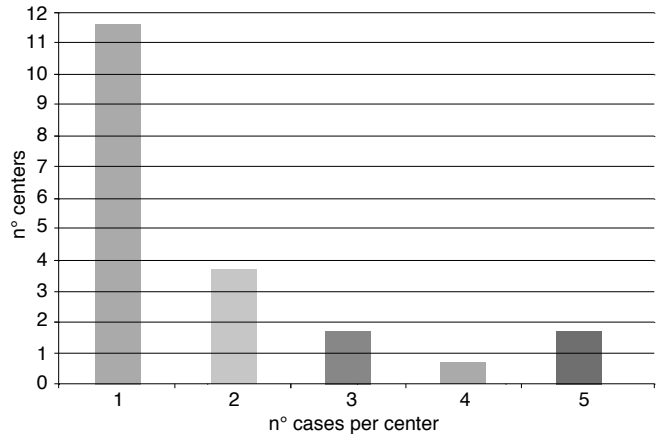


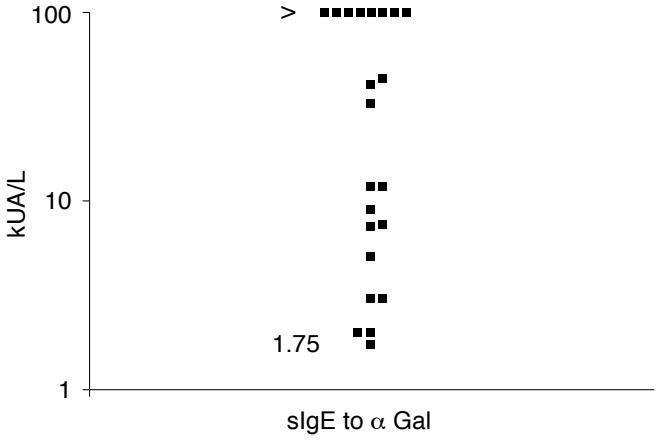
Figure 3 *- Number of cases reported for single center.*



Discussion

This survey shows that the α-Gal syndrome is present throughout all Italy, as expected in view of *Ixodes* spread in the country. Nonetheless, cases reported from Northern Italy exceed those reported from other regions. Whether this really reflects a higher prevalence, or it is due to a more widespread awareness of this syndrome and/or to a larger availability of the technology to detect specific IgE to α-Gal, remains to be established.

Figure 4 *- Values of specific IgE to α-Gal in patients with α-Gal syndrome.*



Only a minority of patients with cetuximab allergy (22.2%) vs 62.5% of those with delayed red meat allergy recalled tick bites. These percentages are lower than those reported by Commins et al. (10), who found that more than 90% of subjects with serum IgE to α-Gal had a history of tick bites, and by Kennedy et al. (22), who reported in a paediatric population with delayed anaphylaxis to red meat a tick exposure in 100% of cases. A possible explanation for this fact might be that, unlike subjects exposed to *Amblyomma americanum*, subjects exposed to *Ixodes ricinus* are not always aware of being bitten, as during the attachment and feeding process *Ixodes ricinus* injects a complex mixture of bioactive chemicals into the host, eventually resulting in a painless bite. Moreover, larval ticks are about 1 mm in diameter and are generally not seen or appear as small black dots, but still may cause sensitization through their bites (10). As expected, the majority of patients were bitten in the hills or mountains between one and twelve months before the appearance of symptoms (**Table II**).

An interesting finding of this survey is that reactions were most frequently reported with beef, which is in contrast with data from other European surveys, where beef and pork showed a similar importance as symptoms elicitors (16). Another important difference with the cases described in Germany and France, is that in Italy most of the reactions were seen after ingestion of butcher's cut meat, whereas in the other European countries mammalian kidney, innards, and sausages (frequently including innards), were the major elicitors (16,17, 23). Thus, the different food habits between European countries may play a relevant

Table 2 *- Features of patients with delayed red meat allergy*

Question	N° (%)
1. Do the patient remember the tick bite	
yes	25/40 (62.5)
no	10/40 (25.0)
unknown	5/40 (12.5)
2. If yes, how many months before the adverse reaction?	
< 1	0/22 (0.0)
1 - 3	5/22 (22.7)
4 - 6	5/22 (22.7)
7 - 12	6/22 (27.3)
> 12	6/22 (27.3)
3. The patient was bitten	
in urban area	0/25 (0.0)
in rural area on the plain	1/25 (4.0)
in the hills	19/25 (76.0)
in the mountains	5/25 (20.0)
4. How many adverse reactions the patient complained before the diagnosis?	
0 - 1	4/38 (10.5)
2 - 4	21/38 (55.3)
> 4	13/38 (34.2)
5. Which kind of meat caused the adverse reaction?	
pork	7/40 (17.5)
beef	30/40 (75.0)
horse	0/40 (0.0)
lamb	2/40 (5.0)
game	1/40 (2.5)
rabbit	0/40 (0.0)
6. Which was the dish preparation?	
butcher's cut	29/40 (72.5)
ham	1/40 (2.5)
sausages	2/40 (5.0)
würstel	0/40 (0.0)
hamburger	1/40 (2.5)
tinned meat	0/40 (0.0)
tripe	4/40 (10.0)
liver / kidney	1/40 (2.5)
other	2/40 (5.0)
7. Adverse reaction with foods/products a part the meat	
no	31/40 (77.5)
yes	9/40 (22.5)
If yes	
milk	3/9 (33.3)
sweet / toffee	4/9 (44.4)
cakes	1/9 (11.1)
vaccines	0/9 (0.0)
gel infusion	1/9 (11.1)
other	0/9 (0.0)
8. Symptoms	
urticaria	26/40 (65.0)
angioedema	1/40 (2.5)
oral / gastrointestinal	8/40 (20.0)
anaphylaxis	5/40 (12.5)
9. Symptom onset	
< 30'	3/38 (7.9)
30' - 1 h	4/38 (10.5)
1 h - 2 h	5/38 (13.1)
2 h - 4 h	23/38 (60.6)
> 4 h	3/38 (7.9)
10. Methods used for the diagnosis	
SPT with commercial extracts	21/40 (52.5)
prick to prick with meat	17/40 (42.5)
prick to prick with liver/kidney	11/40 (27.5)
prick with cetuximab	4/40 (10.0)
BAT	2/40 (5.0)
sIgE to α-Gal	22/40 (55.0)

role in the occurrence of the syndrome. In fact, in many regions of Europe innards are both consumed as local delicacies, and used in sausage products. Kidney, tripe, heart, sweetbread, lung, brain, and tongue from pork, beef, and lamb are processed in such delicacies and consumed in Germany and France. Interestingly, time delay > 2-4 hours was seen in about 70% of Italian subjects, which is typically associated with muscle meat ingestion, whereas the allergic reaction occurs as short as 1 h follow-

ing the consumption of pork kidney (16,17,24), thus behaving like a classic immediate-type allergy probably due to the higher content of α-Gal in kidney (17,25).

According to clinical histories, 65% of α-Gal syndrome patients experienced urticaria, and only 12.5% had potentially dangerous systemic allergic reactions on at least one occasion (anaphylaxis > grade II according to Ring/Messmer) (26). The lower severity of reactions in Italian patients than in those described in American and other European series (16,20) remains an open question, although we cannot rule out a role played by the offending food (muscle meat vs kidney-innards) and the presence / absence of co-factors (that were not investigated in the present survey).

Finally, 22.5% of patients with delayed red meat allergy experienced adverse reactions after consuming foods other than meat, in almost all cases after ingesting milk or gelatin-containing sweets.

In conclusion, α-Gal syndrome is present throughout Italy, although it is mostly diagnosed in the Northern part of the country. Beef meat is the most common offending food, symptoms are not extremely severe in most cases and develop 2-4 hour after the ingestion. Nearly 25% of patients react to foods other than meat, namely milk and gelatin-containing sweets.

Acknowledgments

We thank all the doctors that answered the questionnaire: Antonicelli Leonardo, Ancona; Ariano Renato, Bordighera (IM); Arigliano Luigi, Verona; Atzeni Isabella, S. Gavino Monreale (VS), Avolio Tiziana, Ome (BS); Baldassarre Rossella, Avellino; Bernardi Paola, Padova; Billeri Lucia, Padova; Borrelli Paolo, Aosta; Bramè Barbara, Milano; Buonomo Alessandro, Roma; Caruso Cristiano, Roma; Caruso Maria, Genova; Cremonte Luigi, Novi Ligure (AL); Cucinelli Francesco, Avezzano (AQ); Cutajar Marina, Sorrento; De Cristofaro Maria Laura, Termoli (CB); De Guglielmi Alessandro, Genova; Della Torre Fabrizio, Milano; Di Paolo Camilla, Brescia; Ebbli Antonio, Savona; Esu Stefania, Cagliari; Franchini Maurizio, Jesolo (VE); Gabrielli Anna Rita, Perugia; Genovese Carmelo, Barcellona Pozzo di Gotto (ME); Greco Giacomo, Crotone; Hendrich Birgit, Padova; Iannello Gioacchino, Pontedera (PI); Inciso Giovanni, Meta (NA); Ingrassia Antonino, Marsala; Intravaia Rossella, Catania; Kamberi Erilda, Repubblica di S. Marino; Liccardi Gennaro, Napoli; Lodi Rizzini Fabio, Brescia; Lucania Anna, Palermo; Massironi Franco, Monza; Micucci Corrado, Jesi (AN); Montera Carmen, Salerno; Murzilli Francesco, Avezzano (AQ); Musarra Antonino, Scilla (RC); Natoli Rosalba, Palermo; Niniano Rosanna, Voghera (PV); Parise Giuseppe, Thiene (VI); Pingitore Giuseppe, Roma; Piunti Enrico, S. Benedetto del Tronto (AP); Platzgummer Stefan, Merano (BZ); Rossi Renato, Savigliano (CN); Scalone Gino, Chiaravalle C.le (CZ); Scarantino Giovanna, Caltanissetta; Scarpa Alessandro, Mirano (VE); Tasin Laura, Trento; Uasuf Carmen, Palermo; Zanforlin Mario, Monselice (PD); Zanoni Giovanna, Verona.

References

1. Spiro RG, Bhoyroo VD. Occurrence of apha-D-galactosyl residues in the thyroglobulins from several species. Localization in the saccharide chains of the complex carbohydrate units. J Biol Chem 1984; 259:9858-66.
2. Macher BA, Galili U. The Galalpha 1,3 Galbeta 1,4GlcNAc-R (alpha-Gal) epitope: a carbohydrate of unique evolution and clinical relevance. Biochim, Biophys Acta 2008; 1780:75-88.
3. Koike C, Fung JJ, Geller DA, Kannagi R, Libert T, Luppi P, et al. Molecular basis of evolutionary loss of the alpha 1,3-galactosyltransferase gene in higher primates. J Biol Chem 2002; 22:10114-20.
4. Galili U, Mandrell RE, Hamadeh RM, Shohet SB, Griffiss JM. Interaction between human natural anti-alpha-galactosyl immunoglobulin G and bacteria of the human flora. Infect Immun 1988; 56:1730-7.
5. Galili U. Anti-Gal: an abundant human natural antibody of multiple pathogeneses and clinical benefits. Immunology 2013; 140:1-11.
6. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3 galactose. N Engl J Med 2008; 358:1109-12.
7. Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, et al. Delayed anaphylaxis, angioedema, or urticarial after consumption of red meat in patients with IgE antibodies specific for galactose-α-1,3-galactose. J Allergy Clin Immunol 2009; 123:426-33.
8. Commins SP, Platts-Mills TA. Anaphylaxis syndrome related to a new mammalian cross-reactive carbohydrate determinant. J Allergy Clin Immunol 2009; 123:426-33.
9. Van Nunen SA, O'Connor KS, Clarke LR, Boyle RX, Fernando SL. An association between tick bite reactions and red meat allergy in humans. Med J Aust 2009; 190.510-11.
10. Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-α-1,3-galactose. J Allergy Clin Immunol 2011; 127:1286-93.
11. Hamsten C, Starkhammar M, Tran TAT, Johansson M, Bengtsson U, Ahlen G, et al. Identification of galactose-α-1,3-galactose in the gastrointestinal tract of the tick Ixodes ricinus; possible relationship with red meat allergy. Allergy 2013; 68:549-52.
12. Araujo RN, Franco PF, Rodrigues H, Santos LC, McKay CS, Sanhueza CA, et al. Amblyomma sculptum tick saliva: α-Gal identification, antibody response and possible association with red meat allergy in Brazil. Int J Parassitol 2016; 46:213-20.
13. Chinuki Y, Ishiwata K, Yamaji K, Takahashi H, Morita E. Haemaphysalis longicornis tick bites are a possible cause of red meat allergy in Japan. Allergy 2016; 7:421-5
14. Platts-Mills TA, Schuyler AJ, Hoyt AE, Commins SP. Delayed anaphylaxis involving IgE to galactose-alpha-1,3-galactose. Curr Allergy Asthma Rep 2015; 15:12.
15. Steinke JW, Platts-Mills TA, Commins SP. The alpha-gal story: lesson learned from connecting the dots. J Allergy Clim Immunol 2015; 134:589-96.
16. Fisher J, Yazdi AS, Biedermann T. Clinical spectrum of α-Gal syndrome: from immediate-type to delayed immediate-type reactions to mammalian innards and meat. Allergo J Int 2016; 25-62.

17. Morisset M, Richard C, Atier C, Jacquenet S, Croizier A, Beaudoin E, et al. Anaphylaxis to pork kidney is related to IgE antibodies specific for galactose-α-1,3-galactose. Allergy 2012; 67:699-74.
18. Calamari AM, Poppa M, Villalta D, Pravettoni V. Alpha-Gal anaphylaxis: the first case report in Italy. Eur Ann Allergy Clin Immunol 2015; 47:161-2.
19. Villalta D, Pantarotto L, Da Re M. Conte ME, Sjolander S, Borres MP, Martelli P. High prevalence of sIgE to galactose-α-1,3-galactose in rural pre-Alps area. A cross-sectional study. Clin Exp Allergy 2016; 46:377-80.
20. Corominas M, Gastaminza G, Lobera T. Hypersensitivity reactions to biological drugs. J Investig Allergol Clin Immunol 2014; 24:212-25.
21. Michel S, Scherer K, Heijnen IAFM, Bircher A. Skin prick test and basophil reactivity to cetuximab in patients with IgE to alpha-gal allergy to red meat. Allergy 2014; 69:403-5.
22. Kennedy JL, Stallings AP, Platts-Mills TA, Oliveira WM, Workman L, James HL, et al. Galactose-α-1,3-galactose and delayed anaphylaxis, angioedema, and urticaria in children. Pediatrics 2013; 131:1545-52.
23. Jappe U, Platts-Mills T, Prybilla B, Kreft B, Ludwig A, walker A, et al. IgE reactivity to galactose-α-1,3-galactose: a prospective multicenter study on meat allergy, urticarial and anaphylaxis. XXXI Congress of the European Academy of Allergy and Clinical Immunology abstract Book, Geneva, Switzerland, 16-20 June 2012. Allergy 2012; 67(suppl 96):96.
24. Fisher J, Hebsaker J, Caponetto P, Platts-Mills TA, Biedermann T. Galactose-alpha-1-3-galactose sensitization is a prerequisite for pork-kidney allergy and cofactor-related mammalian meat anaphylaxis. J Allergy Clin Immunol 2014; 34:755-9.
25. Hilger C, Fisher J, Swiontek K, Hentges F, Lehners C, Eberlein B, et al. Two galactose-α-1,3-galactose carrying peptidases from pork kidney mediate anaphylactogenic responses in delayed meat allergy. Allergy 2016; 71:711-9.
26. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet 1977; 1:466-9.

M.C. Verga[1], R. Pastorino[2], A. Casani[3], F. Inturrisi[2], C. de Waure[2], A. Pugliese[4], I. Dello Iacono[5]

Prevalence, molecular characterization, and clinical relevance of sensitization to *Anisakis simplex* in children with sensitization and/or allergy to *Dermatophagoides pteronyssinus*

[1]Primary Care Pediatrics, ASL Salerno, Vietri sul Mare, Italy
[2]Department of Public Health, Section of Hygiene, Università Cattolica del Sacro Cuore, Rome, Italy
[3]Primary Care Pediatrics, ASL BN1, Benevento, Italy
[4]Ismea, Istituto di Servizi per il Mercato Agricolo Alimentare, Rome, Italy
[5]Department of Paediatrics, Fatebenefratelli Hospital, Benevento, Italy

Key words
Anisakis simplex; Dermatophagoides pteronyssinus; prevalence; sensitization; allergy

Corresponding author
Maria Carmen Verga
C.so Umberto I, 103
84019 Vietri sul Mare (SA), Italy
Phone: +39 089 761354
Fax: +39 089 761354
Cell: +39 338 3800 589
E-mail: vergasa@virgilio.it

Doi
10.23822/EurAnnACI.1764-1489.26

Summary
Prevalence of the Anisakis Simplex*'s (AS) sensitization in children sensitized to* Dermatophagoides pteronissynus *(DP) is not known, neither it is to which percentage it might be due to cross-reactivity. The primary objective of the present retrospective cross-sectional study is to evaluate the prevalence of sensitization to AS in children sensitized or allergic to DP. Secondary outcomes were the prevalence of cross-reactivity and clinical relevance of the condition. The prevalence of sensitization to AS differs significantly among patients sensitized and not to DP (13.43% vs. 3.80%; p=0.019). The higher prevalence is mainly due to cross-reactivity with Der p10 (OR=8.86; 95% CI=4.33–40.74; p=0.0001). Currently, the sensitization to AS seems to have no clinical relevance in the pediatric population.*

Introduction

The nematode *Anisakis simplex* (AS) is a common parasite of fish, marine mammals and invertebrates. The life cycle comprises the stage of egg, various stages of larva and adult nematode. The encapsulated larvae of the third stage are common in fish organs and muscles. They infest fish of various species over large geographical areas. Eating raw or undercooked fish can result in infestation with the larva and can cause severe gastrointestinal symptoms (anisakiasis). Several studies suggest that AS can be a food allergen and induce IgE-mediated reactions such as urticaria, angioedema, and anaphylaxis. AS can cause allergic reactions even when well cooked, because some allergens are thermostable. There have been reports of occupational allergy with asthma and conjunctivitis, and a causal relationship was demonstrated between AS and chronic urticaria (1). Finally, AS can also be responsible for delayed, cell-mediated allergic reactions, specifically eczema, caused by live, cooked, and frozen larvae after repeated handling of raw fish (2).

In recent years there has been an increase in cases of anisakiasis probably due to several factors: increase in the number of sea mammals, "global cuisine", faster cooking methods (microwave) also to preserve vitamins, increase in fish consumption for healthier lifestyles (3).

About 90% of the cases worldwide occurs in Japan, followed by Italy, Spain, Hawaii, and other countries where raw or undercooked fish consumption is a tradition. The prevalence of sensitization in Norway, where fish is eaten baked or fried, is around 2%, while in Spain and Japan is 12% (4,5,6). The hypersensitivity, as well as the susceptibility to the disease, may be explained by genetic predisposition (7).

Clinical manifestations of AS are essentially gastric, intestinal, ectopic and systemic disorders, probably according to the route of sensitization (8).

The prevalence of sensitization in Italy is on average 4.5%, with significant geographical differences (variation from 0.4% to 12.7%) due to local culinary traditions, being greater on the Tyrrhenian and Adriatic coasts where it is common to eat raw and marinated fish. In the mainland, the sensitization to AS is essentially related to migration: in Milan and Turin 60% of people sensitized to AS is native to Southern Italy and non-EU countries (9).

The cause of sensitization is not clear. It has been suggested that sensitization in asymptomatic individuals is due to early infestations. However, cross-reactivity may also be a possible explanation, demonstrated between AS and other nematodes, arthropods (eg. *Blatta germanica*) and some types of dust mite (*Acarus siro, Lepidoglyphus destructor, Tyrophagus putrescentiae*, DP) (10). In a recent study of adult patients in Sicily, a region in which the consumption of raw or marinated fish is very common, the co-sensitization to dust mites results in an OR of 1.98 for AS allergy (9).

The cross-reactivity is due to a thermostable protein, highly conserved, present in muscle and non-muscle cells: the tropomyosin. This protein is the major allergen of shellfish, and it is the main allergen responsible for the molecular and clinical cross-reactivity by ingestion of shellfish and by inhalation of other invertebrates, such as dust mites and insects (11). Actin and myosin together have an important role in contractile activity, i.e. motility adjustment and cell morphology (12). Der p10 (DP), Ani s3 (AS) and Pen a1 (shrimp) are among the tropomyosins currently identified (11). In a subject sensitized to multiple allergens, eg. DP and AS, the presence of IgEs against tropomyosin and not against the genuine allergens is an indication of cross-reactivity. The sensitization to tropomyosin may involve allergic reactions of highly variable severity, with very low clinical impact until anaphylaxis (11).

Data on the prevalence of sensitization to AS in the Italian pediatric population are limited. A 2001 study on patients consecutively referred to an Allergology Center, reported a prevalence of 6.1%. The sensitized patients had no allergic symptoms to AS. The sensitization was significantly associated with sensitization to DP and other allergens such as cod and soia (13).

The importance of cross-reactivity in patients sensitized to the AS, in particular in the pediatric age, is not known, both with respect to the immunological profile (prevalence of cross-reactivity or sensitization to genuine allergens) and to the clinical manifestations (allergic reactions or asymptomatic sensitization). Therefore, the primary objective of this study is to assess the prevalence of sensitization to the AS in children sensitized and/or allergic to DP.

Secondary endpoints are:

1. to evaluate the cross-reactivity between AS and DP
2. to assess the clinical relevance of sensitization to AS in children sensitized and/or allergic to DP (presence and severity of allergic reactions following ingestion / inhalation of AS).

Methods

We conducted a retrospective cross-sectional study by extracting and analyzing data from the medical records of a specific sub-population of patients referring to the Paediatric Allergy Day Hospital (DH) of the Sacro Cuore di Gesù Fatebenefratelli Hospital in Benevento (Italy), from January 1st 2013 to October 30th 2016. Every year, about 500 patients refer to the Pediatric Allergy DH, for a total of about 1800 medical records during the period of the study. Of these, 294 were selected, on the basis of inclusion and exclusion criteria.

Inclusion criteria. Pediatric patients (1 - 18 years) with suspected sensitization or allergy to DP, who underwent allergy testing for DP and AS, and refer consecutively to the Paediatric Allergy DH of the Fatebenefratelli Hospital in Benevento. *Exclusion criteria*. Patients who do not meet the inclusion criteria, with suspicion of allergy not related to the DP, or who meet the inclusion criteria but have malformations or are suffering from severe chronic illness, or assume antihistamines, corticosteroids or immunosuppressants. Data regarding medical history, Skin prick tests (SPT) and serum IgEs were extracted from the medical records of the included patients.

Medical history was collected by the referent of the Paediatric Allergy DH of the Fatebenefratelli Hospital in Benevento, with specific reference to respiratory allergic symptoms, food allergy and allergy to DP. Skin prick tests (SPT) were performed with commercial extracts for *Dermatophagoides farinae* (DF), *Dermatophagoides pteronyssinus* (DP), *Anisakis*, cockroach, shrimp, (Alk-Abello by International Pharmaceutical Immunology, SA, Madrid, Spain: protein concentration =1 mg/ml). The SPT results were considered positive (wheal diameter >3 mm) in line with the recommendations of the European Academy of Allergology and Clinical Immunology (15). Serum IgEs against DF, DP, Der p1, p2 Der, Der p10, Pen a1 (Immuno CAP Phadia) were measured. Values >0.35 KU/L were considered positive (13).

Outcomes

Primary outcome. Prevalence of STP positivity (sensitization) to AS among patients positive to the DP. *Secondary outcomes*. Cross-reactivity (associations with sensitization to cockroach, shrimp, tropomyosins Der p10 and Pen a1) and clinical relevance (suggestive clinical history of allergic reactions occurring some minutes up to 2 hours after ingestion of raw or marinated fish, confirmed by a positive SPT to AS) of sensitization to AS.

Data collection

All the data relevant for the study were collected using an ad hoc database in Excel (Microsoft software).

Statistical analysis

Sample size. For an expected prevalence of sensitization to AS=6%, based on the data already known in the literature (12), with the probability alpha=0.05 and beta=0.20, with a precision=4%, the minimum sample size calculated was equal to 140 patients.

Statistical tests. Mean±standard deviation (SD), median and range (interquartile range, minimum and maximum) were calculated for continuous variables, whereas proportions (prevalences) were calculated for categorical variables. To evaluate differences between frequencies, χ_2 test was used. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated between some variables (DP, Der p10, Shrimp, Pen a1 sensitization, DP allergy, Food allergy) and AS sensitization. The p value <0.05 was considered statistically significant. For statistical analysis STATA 13 software was used. The sample size was calculated with StatCalc of EpiInfo ver. 7.2.

Results

A total of 294 patients were selected, with a mean age of 7.7±3.5 years (median=7; range=0–16). Data on sensitization and discharge diagnoses are summarized in **Table I**.

The prevalence of SPT positivity to the tested allergens was: *Dermatophagoides farinae* 68.4%, DP 69.4%, AS 10.5%, cockroach 8.2%, shrimp 2.4%, Der p1 43.2%, Der p2 38.8%, Der p10 5.8%, Pen a1 5.1%. IgEs titer (KU/L) against DP and tropomyosins Der p10 and Pen a1 were respectively 19 (0– >100), 0.01 (0–37), and 0.02 (0–38.4)

Table I *- Age, sensitization (IgEs; SPT) and allergies (allergy to DP, food allergy).*

Variable	N°	Positives N° (%)	Mean (SD)	Median (interquartile; range min–max)
Age (years)	254		7.67 (3.54)	7 (5; 0–16)
Dermatophagoides farinae (DF) IgEs (KUA/L)	177	140 (79.10)	28.17 (34.08)	13.3 (42.67; 0– >100)
Dermatophagoides pteronyssinus (DP) IgEs (KUA/L)	183	153 (83.61)	39.05 (41.32)	19 (92.00; 0– >100)
Der p1 IgEs (KUA/L)	289	127 (43.94)	27.63 (35.33)	5.52 (70.67; 0– >100)
Der p2 IgEs (KUA/L)	232	114 (49.14)	26.12 (35.33)	2.59 (53.07; 0– >100)
Der p10 IgEs (KUA/L)	226	16 (7.08)	0.94 (4.82)	0.01 (0.06; 0–37)
Pen a1 IgEs (KUA/L)	110	15 (13.64)	1.23 (5.12)	0.02 (0.01; 0–38.4)
DF SPT	282	201 (71.28)		
DP SPT	283	204 (72.08)		
Anisakis simplex (AS) SPT	281	31 (11.03)		
Cockroach SPT	281	24 (8.54)		
Shrimp SPT	204	7 (3.43)		
Food Allergy	294	27 (9.18)		
DP Allergy	291	126 (43.30)		

Table II *- Comparison between patients sensitized and not sensitized to Anisakis Simplex.*

		Patients not sensitized to Anisakis simplex N° (%)	Patients sensitized to Anisakis simplex N° (%)	OR (95% IC)	P value[1]
Dermatophagoides pteronyssinus (DP) sensitization	yes	174 (86.57)	27 (13.43)	3.93 (1.15–20.78)	**0.019**
	no	76 (96.20)	3 (3.80)		
Der p10 sensitization	yes	8 (50.00)	8 (50.00)	8.86 (2.56–29.82)	**<0.0001**
	no	186 (93.00)	14 (7.00)		
Shrimp sensitization	yes	1 (16.67)	17 (8.63)	52.94 (5.25–2516.05)	**<0.0001**
	no	180 (91.37)	5 (83.33)		
Pen a1 sensitization	yes	8 (53.33)	7 (46.67)	11 (2.51–46.79)	**<0.0001**
	no	88 (92.63)	7 (7.37)		
DP allergy	yes	105 (84.68)	19 (15.32)	2.35 (1.01–5.70)	**0.028**
	no	143 (92.86)	11 (7.14)		
Food allergy	yes	23 (88.46)	3 (11.54)	1.06 (0.19–3.85)	**0.931**
	no	227 (89.02)	28 (10.98)		

[1]In bold statistically significant results.

With regards to the discharge diagnosis, the prevalence of DP allergy and food allergies was 43% and 9%, respectively.

No patients had allergic symptoms to AS. The prevalence of sensitization to AS, in the subgroups with DP sensitization and allergy, was (**Table II**):

- 13.4% and 3.8% in patients with and without sensitization to DP, respectively
- 50% and 7%, in patients with and without sensitization to Der p10, respectively
- 15.3% and 7.1%, in patients with and without DP allergy, respectively

Sensitization to AS was not associated with age [OR (95% CI)=1.09 (0.98–1.22); p=0.113]. Associations were found with sensitization to DP [OR (95% CI)=3.93 (1.15–13.35); p=0.028)], DP allergy [OR (95% CI)=2.35 (1.07–5.15); p=0.033)], tropomyosin Der p10 [OR (95% CI)=8.86 (3.01–26.1); p<0.0001)], shrimp [OR (95% CI)=52.94 (5.84–479.7); p<0.0001], and tropomyosin Pen a1 [OR (95% CI)=11 (3.08–38.30); p<0.0001] (**Table II**).

Discussion

Sensitization to AS is still under investigation in many clinical, epidemiological and laboratory-based studies aiming at the molecular characterization of the allergens. The latest Italian data are on 3,419 adult patients referred to the Catania University Allergy Center (9). In this sample, the prevalence of sensitization to AS was about 15%, similar to the one recorded in other coastal areas of Italy (~12%), with the only unexplained exception of Messina (0.8%) (1).

Approximately 30% of sensitized patients showed a single sensitization. The age was a risk factor for sensitization to AS, more common in older patients, along with the sensitization to dust mite and mildew.

Despite the reported association between sensitization to DP and to AS, the authors have neither quantified the cross-reactivity, nor they have been able to correlate the AS allergy (for most gastrointestinal and respiratory symptoms, but also, in approximately 7% of cases, for anaphylaxis) with cross-reactivity or sensitization.

In the first study on pediatric patients published in Italy, patients consecutively referred to the Pediatric Allergy Centre of the University of Florence. The sensitization to AS resulted associated with DP and Alternaria sensitization, atopy, allergy to soya, cod and, weakly but significantly, age. The prevalence of sensitization was 6.1%; none of the sensitized patients showed allergic reactions to AS (13).

Another recent study assessed the AS sensitivity in 443 Italian children living in an endemic area, consecutively presenting at three Pediatric Allergy Centers in Rome and one Center in Naples (14). The prevalence of sensitization was 4.5%, and the *Anisakis*-sensitized children were significantly older than control

children; none showed gastrointestinal symptoms or allergic reactions to AS or fish. The authors came to the conclusion that in endemic areas the sensitization to AS is equally frequent in children and adults. Data on the cross-reactivity were not reported. On the basis of these documented associations, with the present study we firstly wanted to quantify the prevalence of sensitization to AS in patients sensitized and not to DP, which resulted being 13.4% and 3.8% (p=0.028), respectively. The low prevalence (3.8%) in non-sensitized to DP is in line with expectations, considering that those patients generally live in the mainland and non-coastal areas, which do not have a traditional habit to consume raw or marinated fish.

Similarly, in patients with allergic symptoms to dust mite, the prevalence of sensitization to AS (15.3%) was significantly higher than in non-allergic patients (7.1%; p=0.033). The high prevalence recorded in sensitized/allergic patients to DP was strongly suggestive of the importance of cross-reactivity, confirmed by the results of the regression analysis: there is a statistically significant association with sensitization, DP allergy, and especially with positivity to tropomyosin Der p10, DP pan allergen (OR=8.86; 95% CI =3.01–5.26, p<0.0001).

Another fact in favor of cross-reactivity is the strong association with the positivity of sensitization to shrimp tropomyosin and its Pen a1 in asymptomatic patients for allergy to this crustacean.

Although the identity of amino acid sequences between Der p10 and Anis s3 is not proven, contemporary positivity of sensitization to AS and DP with Der p10 positivity is considered an expression of cross-reactivity. Recently, identity of amino acid sequences of some AS proteins and other homologous DP allergens (Der p4, Der p8, Der p14, Der p15, Der p18, Der p20) have been demonstrated, although they are not yet available for allergy diagnosis in clinical practice (3).

Based on these new acquisitions, the sensitization to AS found in 7% of Der p10 negative patients cannot definitely be attributed to genuine allergens, but it is likely that a proportion of this sensitivity is also due to cross-reactivity with antigens other than Der p10.

Despite the strength of the association, from our results it is not possible to consider the sensitization to Der p10 as certainly predictive of sensitization to AS, so that, in absence of further data, it is necessary to assess sensitization to AS with specific allergy tests, when indicated.

Contrary to previous publications and according to our results, sensitization to AS is neither associated with age, nor with concomitant food allergies, but the small number of cases does not allow us to determine if those are real data due to different epidemiological conditions, or if they are due to a statistical beta error (false negative).

In our study, as in that of Bernardini et al. (13), patients sensitized to AS did not have symptoms of allergic reactions to AS, so the clinical relevance of the sensitization and the related cross-reactivity with DP is, at least in the pediatric age, apparently inexistent.

A strength of our study is the demonstration, in patients sensitized to DP and AS, of the importance of cross-reactivity, also confirmed by the results of molecular investigations, specifically by dosages of Tropomyosins Der p10 and Pen a1.

The limits are as follows: we have no data on recombinant allergen-specific IgE Anis s3, and we do not know if this result could be confirmed in larger or different populations, or if it could be confirmed on the same patients with an adequate follow-up. The studies on adults published to date do not resolve the issue.

Conclusions

This study shows that sensitization, in patients asymptomatic for AS allergy but sensitized to DP, is essentially due to cross-reactivity and is associated with positivity of tropomyosin Der p10, DP pan allergen. Currently, the sensitization to AS seems to have no clinical relevance in the pediatric population, since it does not entail an increased risk of allergic reaction to AS, but we do not know if the risk increases with age, and if it may change in relation to genetic and/or environmental factors. Therefore, further studies are needed to confirm these results, as well as to complete the identification and characterization of AS allergens and the counterparts in nematodes, insects and crustaceans.

References

1. AAITO-IFIACI Anisakis Consortium. Anisakis hypersensitivity in Italy: prevalence and clinical features: a multicenter study. Allergy 2011; 66(12):1563-9.
2. Ventura MT, Tummolo RA, Di Leo E, D' Erasmo M, Arsieni A. Immediate and cell-mediated reactions in parasitic infections by Anisakis simplex. J Investig Allergol Clin Immunol 2008; 18:253-9.
3. Fæste CK, Jonscher KR, Dooper MM. Characterisation of potential novel allergens in the fish parasite Anisakis simplex. EuPA Open Proteom 2014; 4:140-55.
4. Del Pozo MD, Moneo I, de Corres LF, Audicana MT, Muñoz D, Fernandez E, et al. Laboratory determinations in Anisakis simplex allergy. J Allergy Clin Immunol 1996; 97:977-84.
5. García M, Moneo I, Audicana MT, del Pozo MD, Muñoz D, Fernández E, et al. The use of IgE immunoblotting as a diagnostic tool in Anisakis simplex allergy. J Allergy Clin Immunol 1997; 99:497-501.
6. Hwang YK, Kim JS, Lee JB, Song TJ, Joo KW, Lee JS, et al. Human anisakiasis: diversity in antibody response profiles to the changing antigens in larval excretions / secretions. Parasite Immunol 2003; 25:1-7.
7. Sánchez-Velasco P, Mendizábal L, Antón EM, Ocejo-Vinyals G, Jerez J, Leyva-Cobián F. Association of hypersensitivity to the nematode Anisakis simplex with HLA class II DRB1*1502-DQB1*0601 haplotype. Hum Immunol 2000; 61:314-9.

8. Armentia A, Martin-Gil FJ, Pascual C, Martín-Esteban M, Callejo A, Martínez C. Anisakis simplex allergy after eating chicken meat. J Investig Allergol Clin Immunol 2006; 16:258-63.
9. Heffler E, Sberna ME, Sichili S, et al. High prevalence of Anisakis simplex hypersensitivity and allergy in Sicily, Italy. Ann Allergy Asthma Immunol 2016; 116(2):146-50.
10. Bernardini R, Mistrello G, Novembre E, et al. Cross-reactivity between IgE-binding proteins from Anisakis simplex and Dermatophagoides pteronyssinus. Int J Immunopathol Pharmacol 2005; 18(4):671-5.
11. La Grutta S., Calvani M., Bergamini M., Pucci N., Asero R. Allergia alla Tropomiosina: dalla diagnosi molecolare alla pratica clinica. Rivista di Immunologia e Allergologia Pediatrica 2011; (2):20-38.
12. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, et al. EAACI Molecular Allergology User's Guide. Pediatr Allergy Immunol 2016; 27 Suppl 23:1-250.
13. Bernardini R, Lombardi E, Novembre E, et al. Predictors of Anisakis simplex symptoms. Allergy 2000; 55(10):979-80.
14. Tripodi S, Pingitore G, Calvani M, Scala G, Rodriguez-Perez R, Sfika I, Asero R. Anisakis Sensitivity in Italian Children: A Prospective Study. J Investig Allergol Clin Immunol 2017; 27:142-3.
15. Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. EAACI Food Allergy and Anaphylaxis Guidelines Group. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy 2014; 69(8):1008-25.

ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 49, N 6, 276-280, 2017

M. Al-Ahmad[1,2], T. Rodriguez Bouza[2]

Pattern of inpatient referrals to a drug allergy unit in Kuwait

[1]Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait
[2]Drug Allergy Unit, Department of Allergy, Al-Rashed Allergy Center, Ministry of Health, Kuwait

Key words
drug hypersensitivity; inpatients; Kuwait; referral and consultation

Corresponding author
Mona Al-Ahmad
Department of Microbiology
Faculty of Medicine, Kuwait University
P.O. Box 24923, Safat 13110, Kuwait
Phone: +965-24636515
Fax: +965-25332719
E-mail: Alahmadm@hsc.edu.kw

Doi
10.23822/EurAnnACI.1764-1489.18

Summary
Introduction. *There is no information regarding the pattern of inpatient referrals to drug allergy units in Kuwait.* ***Objectives.*** *The main goal of this study is to clarify the pattern of inpatient referrals to a drug allergy unit in terms of incidence, drugs implicated and allergy evaluation outcomes in comparison with studies in other countries.* ***Patients and Methods.*** *A retrospective chart review of inpatient drug allergy consultations at Al-Rashed Allergy Center over a 3-year period was performed.* ***Results.*** *A total of 51 patients were referred for drug allergy consultations, with an estimated incidence of reported drug allergy among inpatients of 0.008%. There is an increasing trend of referrals from public health centres located in proximity to Al-Rashed Allergy Center. Beta-lactams, contrast media, and general anaesthetics were the most common drugs leading to referrals. In total, 30% of patients were diagnosed with an allergy to the offending drug after a full allergy evaluation.* ***Conclusion.*** *Inpatient drug allergy referrals are highly underreported in Kuwait.*

Introduction

There are few studies regarding inpatient consultations for allergy and immunology (1-3), and data are scarce when limited to inpatient drug allergy consultations. Adverse drug reactions (ADRs) have been reported in up to 10-20% of patients, with up to one-third of these reactions being allergic or pseudo-allergic in nature (3-5). Previous studies reported an incidence of drug allergic reactions among inpatients from 0.42% to 0.87% (3-5), with referral rates from 0.078% to 0.231% (3,4), but there are no data regarding Gulf-area countries, such as Kuwait. As the problem of drug allergies is growing worldwide, we sought to investigate referrals for drug allergies to clarify the pattern of inpatient consultations at our drug allergy unit in Al-Rashed Allergy Center in terms of incidence, related drugs and allergy evaluation outcomes.

Materials and methods

Kuwait is divided into six governorates, which are served by six major general hospitals. Al-Rashed Allergy Center is located in the Sabah Hospital Area (in the capital area), and it serves all governorates in Kuwait.

Drug hypersensitivity reactions from all hospitals in Kuwait are referred to our center. This is a retrospective, descriptive study looking at all records from our referrals for inpatient drug allergies. Anaphylaxis has been defined as a severe, life-threatening generalized or systemic hypersensitivity reaction (6). Patients were managed following EAACI guidelines in the case of beta-lactams (7,8), RCM (9) and anaesthesia (10). Skin test concentration were obtained from EAACI guidelines (11) and alternatively from AAAAI practice parameter (12) for the cases not included in EAACI guidelines.

The data were collected for a 3-year period from patients referred to our center, from March 2010 to February 2013, and were reviewed for demographics, drugs implicated and allergy evaluation procedures and outcomes. Epidemiologic data were obtained from January 2011 to December 2013 from the Kuwait Ministry of Health (MOH) Statistics. Ethical approval was obtained from the institutional ethics committee (13-15). Clearance of the study protocol was obtained from the MOH Standing Committee for the Coordination of Health and Medical Research.

The data were coded, entered and analysed using SPSS version 20 software for Windows (IBM, Armonk, NY, USA).

Results

The study included 51 patients and a total of 53 drug consultations. The mean age was 41.69 years, with a range of 6 to 87 years, including 41 females and 10 males.

A total of fifteen referrals were to evaluate allergy to beta-lactams, nine to contrast media, six to anaesthesia, five to antituberculosis drugs, six to NSAIDs, two to carbamazepine, two to aminoglycosides and two to quinolones. Among the remaining six referrals, the offending drugs were protamine, allopurinol, clarithromycin, Vitamin D, hydrocortisone, and local antiseptics.

Unfortunately, eight consultations regarding beta-lactams and one each regarding antituberculosis drugs, quinolones and contrast

Table 1 *- Reactions confirmed by the drug allergy evaluation.*

#	Age	Sex	Initial reaction	Drug eliciting response	Diagnostic evaluation	Comments
1	31	F	Anaphylaxis	General anesthesia	Positive ST Cisatracurium	Alternative skin test negative drug was used
2	26	F	Anaphylaxis	General anesthesia	Positive ST rocuronium, pancuronium	Alternative skin test negative drug was used
3	73	M	Anaphylaxis	RCM	Negative ST Positive clinical history only[1]	Anaphylaxis despite premedication[2]
4	59	F	Anaphylaxis	Carbamazepine	Positive clinical history only[1]	Alternative drug used, no further test needed
5	67	F	Urticaria	Betalactams	Positive ST	Alternative antibiotic used
6	37	M	Urticaria	Betalactams	Positive ST	Alternative antibiotic used
7	63	F	Urticaria Angioedema	Hydrocortisone	Positive ST	Challenge negative for dexamethasone
8	65	F	Urticaria	NSAIDs	Positive challenge	Reaction during desensitization. sPatient tolerated ASA 81 mg
9	59	M	Maculopapular	Allopurinol	Positive patch test	Unsuccessful desensitization
10	52	F	Maculopapular	Antituberculosis	Positive patch test isoniazid, rifampicin	Alternative antibiotic used
11	70	F	Maculopapular	Antituberculosis	Positive clinical history only[1]	Negative culture for TBC, so no further test needed
12	56	F	Maculopapular	RCM	Negative ST Positive clinical history only[1]	Maculopapular rash despite premedication[2]
13	23	F	DRESS	Carbamazepine	Positive clinical history only[1]	Alternative drug used, no further test needed
14	11	M	DRESS	Betalactams	Positive clinical history only[1]	Alternative drug used, no further test needed
15	29	F	DRESS	Antituberculosis	Positive clinical history only[1]	Alternative drug used, no further test needed
16	30	F	DRESS	Antituberculosis	Positive clinical history only[1]	Alternative drug used, no further test needed

[1]A positive clinical history was present in all cases. However, "only" was highlighted if a positive clinical history was used by itself for diagnosis.
[2]Premedication indicated as per EAACI guidelines (3).

media were lost to follow-up. Among the forty-two patients who completed allergy evaluations, ten were confirmed to have drug allergies after full allergy evaluations and diagnostic workups, whereas six patients (two to carbamazepine and one each to beta-lactams, antituberculosis drugs, radiocontrast media and hydrocortisone) were deemed positive from clinical history alone. The remaining twenty-six patients were negative for drug allergy after full evaluations. Consultation for two different drugs in a single patient was presented, once for beta-lactams and quinolones and once for aminoglycosides and quinolones. A total of five patients presented with anaphylactic reactions, including two patients with reactions to neuroblocking agents, one with a reaction to RCM, one with a reaction attributed to Vitamin D and one attributed to carbamazepine. A total of 16/53 (30%) reactions were deemed positive for drug allergy evaluation and they are summarized in **table 1**.

There were increased numbers of referrals from the pulmonary department and Sabah Hospital, located in close proximity to Al-Rashed Allergy Center (**table 2**). In the Sabah area, we found an average of 161,949 admissions per year for 3932 beds in general hospitals and 56,009 admissions per year for 2653 beds in specialty hospitals. Given an average of 17 patients referred per year during our study period, the estimated incidence of drug allergy among inpatient referrals based on 2011-2013 total hospital admissions is hence reported as 0.008% (**tables 2** and **3**).

Discussion

The pattern of inpatient referrals has significant variability among different studies, with beta-lactams as the most frequently implicated group of drugs for referrals, followed by sulphonamides and antiepileptic drugs (5,16). In our study, the leading referred drugs for evaluation were beta-lactams, contrast media, and general anaesthetics.

We had an increased number of consultation for female patients, which is similar to other studies (2-5). One patient presented anaphylaxis immediately after carbamazepine intake, but skin testing other than patch is not standardized, and a confirmatory diagnostic work-up was not performed due to the availability of alternative medication. We decided to perform desensitization to allopurinol due to the non-severe nature of the previous reaction and the non available alternative (17), a previously published protocol (18) proved unsuccessful for this patient, due to continue flares of maculopapular rash. In 37/53 (70%) reactions the patients were allowed to continue the offending medication as inpatient, and in 16/53 (30%) of cases the patients were deemed as allergic to the offending drug. Among this 16 reactions, in 13 (81%) a suitable alternative was found after cooperation between drug allergy unit and the refereeing consultant, and in 2 (12%) cases who reacted to RCM, either desensitization or increased premedication was recommended for a safe administration of the drug on future exposures. This data supports the potential benefit of increasing the number of referrals to drug allergy units.

The increased referrals for antituberculosis drugs may be explained by close collaboration with and physical proximity to the pulmonary department (**table 3**). We also observed an increased number of consultations from hospitals located in our

Table 2 *- Number of inpatient admissions and consultations among different specialty hospitals in Kuwait.*

Specialty Hospital	**Admissions per year[1]**				**Referrals (3 years)**	**Referrals per 100 admissions / year**
	2011 (1)	**2012 (2)**	**2013 (3)**	**Mean**		
Allergy Center	538	320	335	398	7	0.396
Chest Diseases	7520	7733	7723	7659	7	0.030
Neurology	12118	12087	12267	12157	4	0.011
Kuwait Cancer Center	3339	3506	3423	3423	2	0.020
Psychiatry	3936	3748	3525	3736	2	0.018
Orthopaedics/Trauma	5606	5531	5642	5593	2	0.012
Other Specialized[2]	23144	22921	23065	23043	0	0.000
Total	56201	55846	55980	56009	24	0.014

[1]Data from 2011-13

[2]Infectious Diseases, Maternity and Rehabilitation

1. Department of health information and medical reports Moh, State of Kuwait. Health, Kuwait 2011. Kuwait2011.
2. Department of health information and medical reports Moh, State of Kuwait. Title: Health, Kuwait 2012. Kuwait2012.
3. Department of health information and medical reports Moh, State of Kuwait: Health, Kuwait 2013; 2013.

Table 3 *- Number of inpatient admissions and consultations among different General hospitals in Kuwait.*

General Hospitals	Admissions				Referrals (3 years)	Average referral per 100 admission In 1 year	Distance (Km)[2]
	2011 (1)	2012 (2)	2013 (3)	Average			
Amiri	16537	16406	16859	16601	12[1]	0.024	12
Sabah	18619	16179	16565	17121	8	0.016	-
Mubarak	20243	20681	21148	20691	3	0.005	15
Adan	35129	37113	39020	37087	3	0.003	37
Farwaniya	34587	34439	35921	34982	1	0.001	15
Jahra	35342	35409	35650	35467	0	0.000	27
Total	160457	160227	165163	161949	27	0.006	-

[1]9 in 2013

[2]Distances from different hospitals in Kuwait to Sabah area (Al-Rashed allergy center).

1. Department of health information and medical reports Moh, State of Kuwait. Health Kuwait 2011; 2011.
2. Department of health information and medical reports Moh, State of Kuwait. Health Kuwait 2012; 2012.
3. Department of health information and medical reports Moh, State of Kuwait: Health, Kuwait 2013; 2013.

medical area (Sabah), as well as a decreased number of consultations from distant areas (**table 1**). Interestingly, five out of six salicylate referrals occurred in the final year of the study, and all were requests for aspirin challenge / desensitization by cardiology intensive care units prior to percutaneous intervention. This emphasizes the importance of other departments having knowledge of the services offered by drug allergy units.

In an 18-month-long study in the USA (4) with 36,653 hospitalizations, a 2% incidence of ADR was observed, and antihistamines were administered to 32.7% of this group, with an incidence of 0.65% of suspected allergic reactions per hospitalization. Interestingly, only 12.6% of ADRs were reported by a staff physician, and the remaining were obtained via an electronic review of records. In a 2-year study in Singapore (3) covering 90,910 hospitalizations, a 0.42% estimated incidence of drug allergy was calculated after a random review of electronic records, with only a 0.23% incidence of reported drug allergy. In a study in Korea (5) with 55,432 admissions, a mandatory system of reporting drug hypersensitivity was introduced, and a 4.84% incidence of ADR was found, with only 18% identified as drug hypersensitivity reactions after allergist review, compared with a 0.5% incidence of ADR reported prior to the mandatory reporting system. Consultations for allergy and immunology were studied by Otto et al. (2) in a centre with 218 beds and 15,000 admissions / year, and they noted approximately 15.5 consultations / year for ADRs.

In this study, we calculated the incidence of drug allergy referrals as 0.008% among inpatients. This is a significantly lower estimate than those of previous studies in different countries, in which the incidence of drug allergic reactions among inpatients was calculated as 0.42% to 0.87% (3-5), with referral rates of 0.078% to 0.231% (3,4). However, despite the fact that the number of unreported drug allergic reactions among inpatients has not been examined in our study population, we suppose that this population has a low number of referrals compared with the real incidence of allergic reactions among inpatients, based on previous studies from other countries (3-5). Anaphylactic reactions to drugs are reported to be as high as 1.5 per 5000 patients (19), and they should be referred to the allergy department for further workup. The unexpected incidence of 1.66 referrals / year (0.76 per 100,000 inpatients) for anaphylactic reactions, may be explained by underreporting rather than by a low incidence of anaphylactic reactions. Despite we do not have data to support our hypothesis, we are aware that it might be a bias towards consultation of severe reactions while treating doctors usually manage milder reactions by themselves, usually simply avoiding the offending medication and using second-line therapies, even if they are less effective. In light of these data, we suppose that drug allergies among inpatients are underreported in Kuwait.

Drug desensitization allows the re-introduction of first-line medications in allergic patients. There were only six referrals for desensitization during the period of this study, and none of them were for chemotherapeutics, antibiotics or monoclonal antibodies. This was an interesting and rather unexpected finding, particularly given the wealth of evidence supporting good safety and efficacy profiles (20,21).

The field of drug allergies relies almost entirely on referrals from physicians from other specialties and general practitioners. Pa-

tients "labelled" as allergic to beta-lactams are more likely to be treated with broad-spectrum antibiotics, such as vancomycin or quinolones, leading to increased costs, antibiotic resistance, inpatient admission length-of-stay and overall mortality (22).

Our study has a number of limitations. The low number of referrals may be due to the recent establishment of the drug allergy unit in December 2009 and a lack of knowledge among physicians regarding its existence, as proven by the low number of referrals from distant governorates. A systematic review of files would have revealed the frequency of underreported drug allergy reactions in our study populations, but the lack of electronic records makes this task impossible for our staff.

In conclusion, this is the first study showing highly underreported number of referrals for inpatient drug allergy in a Gulf-area country, with a total of 0.008% compared to 0.42% to 0.87% in other studies, and these differences are increased among hospitals distant to our center. We are working to increase the awareness of allergy evaluations of ADRs to assist with providing better health services that meet the standard of care.

All authors contributed equally in the idea, design, data collection and analysis. All authors edited and approved the final version of the manuscript.

References

1. England RW, Ho TC, Napoli DC, Quinn JM. Inpatient consultation of allergy / immunology in a tertiary care setting. Ann Allergy Asthma Immunol. 2003;90(4):393-7.
2. Otto HF, England RW, Quinn JM. Inpatient allergy / immunology consultations in a tertiary care setting. Allergy Asthma Proc. 2010;31(3):244-51.
3. Thong BY, Leong KP, Tang CY, Chng HH. Drug allergy in a general hospital: Results of a novel prospective inpatient reporting system. Ann Allergy Asthma Immunol. 2003;90(3):342-7.
4. Classen DC, Pestotnik SL, Evans RS, Burke JP. Computerized surveillance of adverse drug events in hospital patients. JAMA. 1991;266(20):2847-51.
5. Park CS, Kim TB, Kim SL, Kim JY, Yang KA, Bae YJ, et al. The use of an electronic medical record system for mandatory reporting of drug hypersensitivity reactions has been shown to improve the management of patients in the university hospital in Korea. Pharmacoepidemiol Drug Saf. 2008;17(9):919-25.
6. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol. 2004;113(5):832-6.
7. Blanca M, Romano A, Torres MJ, Fernandez J, Mayorga C, Rodriguez J, et al. Update on the evaluation of hypersensitivity reactions to betalactams. Allergy. 2009;64(2):183-93.
8. Romano A, Blanca M, Torres MJ, Bircher A, Aberer W, Brockow K, et al. Diagnosis of nonimmediate reactions to beta-lactam antibiotics. Allergy. 2004;59(11):1153-60.
9. Brockow K, Christiansen C, Kanny G, Clement O, Barbaud A, Bircher A, et al. Management of hypersensitivity reactions to iodinated contrast media. Allergy. 2005;60(2):150-8.
10. Mertes PM, Malinovsky JM, Jouffroy L, Working Group of the S, Sfa, Aberer W, et al. Reducing the risk of anaphylaxis during anesthesia: 2011 updated guidelines for clinical practice. J Investig Allergol Clin Immunol. 2011;21(6):442-53.
11. Brockow K, Garvey LH, Aberer W, Atanaskovic-Markovic M, Barbaud A, Bilo MB, et al. Skin test concentrations for systemically administered drugs - an ENDA/EAACI Drug Allergy Interest Group position paper. Allergy. 2013;68(6):702-12.
12. Joint Task Force on Practice P, American Academy of Allergy A, Immunology, American College of Allergy A, Immunology, Joint Council of Allergy A, et al. Drug allergy: an updated practice parameter. Ann Allergy Asthma Immunol. 2010;105(4):259-73.
13. Department of health information and medical reports Moh, State of Kuwait. Health Kuwait 2011; 2011.
14. Department of health information and medical reports Moh, State of Kuwait. Health Kuwait 2012; 2012.
15. Department of health information and medical reports Moh, State of Kuwait: Health, kuwait 2013; 2013.
16. Demoly P VM, Gomes ER, Romano A. Epidemiology and causes of drug hypersensitivity. In: WJ. P, editor. Drug Hypersensitivity: Karger; 2007. p. 18-31.
17. Scherer K, Brockow K, Aberer W, Gooi JH, Demoly P, Romano A, et al. Desensitization in delayed drug hypersensitivity reactions -- an EAACI position paper of the Drug Allergy Interest Group. Allergy. 2013;68(7):844-52.
18. Fam AG, Dunne SM, Iazzetta J, Paton TW. Efficacy and safety of desensitization to allopurinol following cutaneous reactions. Arthritis Rheum. 2001;44(1):231-8.
19. Tejedor Alonso MA, Moro MM, Hernandez JE, Mugica Garcia MV, Albelda CV, Ingelmo AR, et al. Incidence of anaphylaxis in hospitalized patients. Int Arch Allergy Immunol. 2011;156(2):212-20.
20. Castells MC, Tennant NM, Sloane DE, Hsu FI, Barrett NA, Hong DI, et al. Hypersensitivity reactions to chemotherapy: outcomes and safety of rapid desensitization in 413 cases. The Journal of allergy and clinical immunology. 2008;122(3):574-80.
21. Legere HJ, 3rd, Palis RI, Rodriguez Bouza T, Uluer AZ, Castells MC. A safe protocol for rapid desensitization in patients with cystic fibrosis and antibiotic hypersensitivity. Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society. 2009;8(6):418-24.
22. Macy E, Contreras R. Health care use and serious infection prevalence associated with penicillin "allergy" in hospitalized patients: A cohort study. The Journal of allergy and clinical immunology. 2014;133(3):790-6.

M. Viñas[1], F. Pineda[2], A. Izquierdo-Domínguez[1], M. Castillo[2], M.J. Castillo[1], N. Hernández[1], M. Ibero[1]

Occupational allergy to Spagulax® *(Plantago ovata seed)*

[1]Servicio de Alergia del Consorci Sanitari de Terrassa, Barcelona, Spain
[2]Departamento de Aplicaciones, Laboratorio Diater, Madrid, Spain

Key words
Plantago ovata; psyllium; nasal challenge test; rhinoconjunctivitis; occupational allergy

Corresponding author
Marta Viñas Domingo
Ctra. Torrebonica s/n, 08227
Terrassa (Barcelona), Spain
Phone: +34 9373 10 007 ext. 1511
E-mail: MVinas@cst.cat

Doi
10.23822/EurAnnACI.1764-1489.21

Summary
We report the case of a 36-year-old male pharmaceutical laboratory worker. On handling Spagulax® sachets whose content is a laxative called Plantago ovata*, he immediately presented rhinoconjunctivitis.* ***Methods.*** *Specific allergy study included SDS-PAGE with Western Blot and specific nasal challenge to Plantago ovata extract.* ***Results.*** *Prick by prick for Spagulax® was negative. Total IgE: 126.5 U/mL. Western Blot recognized two proteins of 15 and 20 kDa in the extract of* Plantago ovata *and three proteins of 15, 18 and 50 kDa in the extract of* Plantago lanceolata. ***Conclusions.*** *We present a case of occupational allergy due to inhalation of and/or contact with* Plantago ovata *seeds.*

Introduction

Plantago ovata is a herb whose small brown seeds look like fleas, hence its name in Greek, *psyllium*. The shells are pulverized and the resulting powder is known as *ispaghula*. This dust disperses very easily through the air when handled, and is a powerful occupational allergen (1). *Psyllium* seeds contain a large amount of mucilage, that expands when it comes into contact with water and becomes very gelatinous, which is why they are used as a volume laxative (2).
Since Ascher (3) described the first case of *Plantago ovata* seed allergy in 1941, multiple occupational allergic reactions (rhinitis and asthma), and anaphylaxis have been reported (1,4). In workers in the pharmaceutical industry, the prevalence of asthma is around 3.6% and sensitization to *Plantago ovata* seeds is 27.9%, based on prick tests and determination of specific IgE (5). In most individuals, sensitization occurred due to inhalation of the powder of *Plantago ovata* seeds in the workplace (5). Sensitization may be asymptomatic or may trigger reactions ranging from rhinitis or asthma to anaphylaxis (6).
Plantago lanceolata is a known aeroallergen in our country that usually produces seasonal rhinoconjunctivitis mediated by IgE (7). Despite the phylogenetic relationship between *Plantago ovata* and *Plantago lanceolata*, most studies suggest a lack of cross-reactivity between them (8,9).

Case report

We report the case of a 36-year-old male pharmaceutical laboratory worker since 2013, who was responsible for handling Spagulax® sachets, whose content is a laxative called *Plantago ovata* to market in France. On march 2016, he referred rhinoconjunctivitis that started thirty minutes later on sealing those sachets of Spagulax®, and improved after administration of a 20 mg oral ebastine tablet. He performed this task for hours only

three or four times a year. Personal history of atopy: allergic to nuts, corn and peach in the form of acute urticaria. He did not refer rhinoconjunctivitis out of his place of work.

Materials and methods

Prick tests were performed based on the predominant aeroallergen batteries in our area and on foods with commercial extracts (Bial Aristegui company, Bilbao, Spain). Total IgE and IgE specific to positive allergens in the skin test were determined by ImmunoCAP (Thermo Fisher Scientific, Phadia, USA). Hydrochloride histamine 10 mg/mL and 0.9% saline were used as positive and negative controls, respectively.
Prick by prick was performed using the Spagulax® sachet provided by the patient.

Allergenic extracts

Proteins were obtained from *Plantago ovata* and *Plantago lanceolata* as follows. *Plantago ovata* seeds (Natupur, Spain) and *Plantago lanceolata* pollen (Pharmalerga, Czech Republic) were weighed, crushed and suspended at 0.25 g/mL in phosphate buffered saline (PBS) [1.37 mM NaCl, 14.7 mM KH_2PO_4, 78.1 mM Na_2HPO_4, 26.8 mM KCl], pH 7.4. The homogenate was magnetically stirred for 30 min at 5 ± 3ºC. The sample was sieved to remove the residues of seeds and the extract was centrifuged at 10,000 g for 15 min at 5 ± 3ºC. The extract was obtained by filtering the soluble fraction through an AP type 20 glass fibre filter (Merck-Millipore™, Darmstadt, Germany), comprising a glass fibre profiler and a 0.8 mm membrane (AP membrane 2009000, Merck-Millipore™, Darmstadt, Germany), and dialyzed against de-ionized water with membranes with a molecular cut-off of 3500 Da (Visking, Iberlabo) for 16 h at 5 ± 3ºC and then stabilized by freeze drying.

SDS PAGE/IgE Western blot

Proteins from *Plantago ovata* and *Plantago lanceolata* were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), according to Laemmli (10) in 15% polyacrylamide gels under reducing conditions. Proteins were visualised by Coomassie Brilliant Blue R-250 staining and electrophoretically transferred to polyvinylidene difluoride (PVDF, Trans-blot turbo™. BIORAD, Hercules, CA, USA). Binding of IgE antibody to allergens was analysed by Western Blot using the patient's serum and anti-human IgE peroxidase conjugate (Southern Biotech, Birmingham, USA). Chemiluminescence detection reagents (Western Lightning® Plus-ECL. Perkin Elmer. Waltham, MA, USA) were added following the manufacturer's instructions. IgE binding bands were identified using the BioRad Diversity database program.

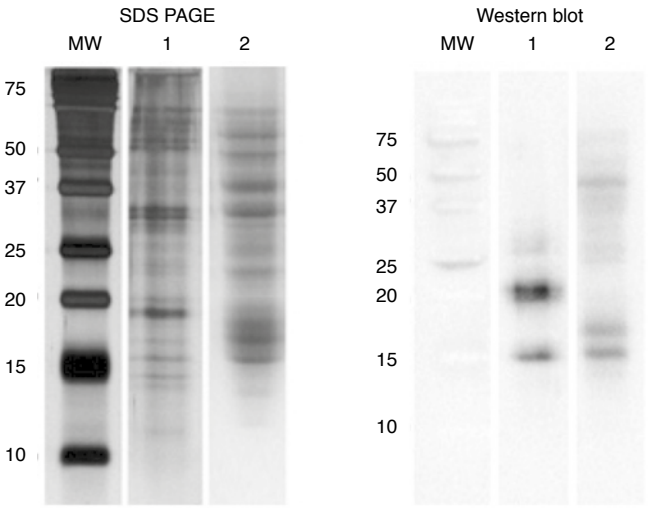
Specific nasal challenge

Specific nasal challenge was performed with *Plantago ovata* extract controlled by an acoustic rhinometer (Rhinometrics SRE 2000) following Rhinoconjunctivitis Committee of the Spanish Society of Allergy and Clinical Immunology recommendation (11). The extract was supplied by Diater laboratories and prepared at two concentrations: 0.107 mg/mL and 1.07 mg/mL. The test was completed by observing a positive response: reduction of nasal volume of between 2 and 6 from the nasal orifice ≥ 20%.

Results

Inhalant skin tests were only positive for house dust mites. Skin test and specific IgE with *Plantago lanceolata* pollen were negative. Food battery tests were positive for nuts (hazelnut, almond and peanut), peach and cereal (rice and corn). Panallergens such as LTP, profilin or polcalcin were negative. Prick by prick to Spagulax® was negative.
Total IgE was 126.5 U/mL and specific IgE to corn: 0.86; rice: 0.23; almond: 0.77; hazelnut: 0.22; peanut: 0.33 and peach: 5.31 kU/L.
Western Blot showed two proteins of 15 and 20 kDa in the *Plantago ovata* extract and three proteins of 15, 18 and 50 kDa in the *Plantago lanceolata* extract, one of which was compatible in molecular weight with Pla a 1 (18 kDA), corresponding to the major allergen (**figure 1**).
Nasal challenge with the *Plantago ovata* extract was positive with a minimum cross area (MCA) and nasal cavity volume 2 cm

Figure 1 *- SDS PAGE and Western blot for specific immunoglobulin E of an extract from a laxative containing Plantago ovata seed and Plantago lanceolata.*



to 6 cm from the nostril = -49% being observed 30 minutes after the last concentration administered (1.07 mg/mL). The symptom score (11) was ≤ 3 points in all tested categories (rhinorrhea, nasal obstruction, nasal itch and sneezing).

Discussion

Plantago ovata is a herb whose seeds are called *psyllium*. The shells are pulverized and the resulting powder is known as *ispaghula*. This dust disperses very easily upon handling and is known to be a potent occupational allergen, that can produce immediate hypersensitivity in exposed workers, especially healthcare workers who dispense it to patients, and workers in the pharmaceutical industries that process the seeds (5,12). Inhalation appears to be the most frequent route of sensitization, but there are also reported cases of anaphylaxis after oral consumption of the laxative or some cereals containing it (2). Our patient developed IgE-mediated rhinoconjunctivitis by inhaling and/or manipulating *Plantago ovata* while packaging it for sale. The frequency of sensitization is high in exposed individuals: 32% of pharmaceutical industry workers have skin tests and/or specific IgE positive for *psyllium* (5). However, the prevalence of occupational asthma in exposed workers is around 3.6-4%, indicating that not all exposed individuals develop the disease; therefore, specific challenge tests are necessary to confirm the diagnosis (5,13).

In our patient, skin tests were negative but the specific nasal challenge was positive, and therefore we confirmed the initial suspicion of occupational rhinoconjunctivitis due to *Plantago ovata*.

Analysis of *Plantago ovata* by immunoblotting showed bands of 15 and 20 kDa, values similar to those reported in previous studies (allergenic proteins oscillate between 10 and 66 kDa) (2,14). To date, no specific immunoblot pattern associated with respiratory or systemic symptoms has been found (15).

Most reports indicate no cross reactivity between *Plantago ovata* and *Plantago lanceolata*, although Bernedo et al. reported the opposite (15). In our case, the patient did not present rhinoconjunctivitis symptoms outside the work place and tests did not demonstrate cross reactivity with *Plantago lanceolata*. Therefore, further studies are warranted.

Conclusions

In conclusion, we report a case of occupational allergy due to inhalation of Spagulax® powder formulated with *Plantago ovata* seeds. Western Blot recognized two proteins with molecular weights of 15 and 20 kDa for *Plantago ovata* and three proteins of 15, 18 and 50 KDa for *Plantago lanceolata*.

References

1. Cartier A, Malo JL, Dolovich J. Occupational asthma in nurses handling psyllium. Clin Allergy. 1987;17:1-6.
2. Alemán AM, Quirce S, Bombín C, Sastre J. Asma relacionada con la inhalación de Plantago ovata. Med Clin (Barc). 2001;116:20-2.
3. Ascher MS. Psyllium seed sensitivity. J Allergy. 1941;12:607-9.
4. Schoenwetter WF, Steinberg P. Psyllium hypersensitivity, nurses and geriatric units. Ann IIntern Med. 1985;103(4):642.
5. Bardy JD, Malo JL, Seguin P, Ghezzo H, Desjardins J, Dolovich J, Cartier A. Occupational asthma and IgE sensitization in a pharmaceutical company processing psyllium. Am Rev Respir Dis. 1987;135(5):1033-8.
6. Lantner RR, Espiritu BR, Zumerchik P, Tobin MC. Anaphylaxis following ingestion of a psyllium-containing cereal. Jama. 1990;264(19):2534-6.
7. Rosenberg S, Landay R, Klotz SD, Fireman P. Serum IgE antibodies to psyllium in individuals allergic to psyllium and English plantain. Ann Allergy. 1982;48(5):294-8.
8. Aleman AM, Quirce S, Bombin C, Sastre J. Asthma related to inhalation of Plantago ovata [in Spanish]. Med Clin (Barc.). 2001;116(1):20-2.
9. Morgan MS, Arlian LG, Vyszenski-Moher DL, Deyo J, Kawabata T, Fernandez-Caldas E. English plantain and psyllium: lack of cross-allergenicity by crossed immunoelectrophoresis. Ann Allergy Asthma Immunol. 1995;75(4):351-9.
10. Laemmli, UK. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227(5259): 680-5.
11. Dordal MT, Lluch-Bernal M, Sánchez MC, et al. Allergen-specific nasal provocation testing: Review by the rhinoconjunctivitis commitee of the Spanish society of allergy and clinical immunology. J Investig Allergol Clin Immunol. 2011;21(1):1-12.
12. Hinojosa M, Dávila I, Zapata C, Subiza J, Cuesta J, Quirce S. Asma ocupacional inducido por polvo de semillas de *Plantago ovata* en trabajadores de la industria farmacéutica. Rev Esp Alergol Inmunol Clin. 1990:5:139-45.
13. Marks GB, Salome CM, Woolcock AJ. Asthma and allergy associated with occupational exposure to ispaghula and senna products in a pharmaceutical work force. Am Rev Respir Dis. 1991;144:1065-9.
14. James JM, Cooke SK, BArnett A, Sampson HA. Anaphylactic reactions to a psyllium-containing cereal. J Allergy Clin Immunol. 1991;88(3 Pt 1):402-8.
15. Bernedo N, Garcia M, Gastaminza G, Fernandez E, Bartolomé B, Algorta J, Muñoz D. Allergy to Laxative Compound (Plantago ovata seed) Among Health Care Professionals. J Invest Allergol Clin Immunol. 2008;Vol.18(3):181-9.

M. Noshela Ghazanfar[1], S.F. Thomsen[1,2]

Transient hair loss in patients with chronic spontaneous urticaria treated with omalizumab

[1]Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark
[2]Center for Medical Research Methodology, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Corresponding author
Misbah Noshela Ghazanfar
Department of Dermatology
Bispebjerg Hospital
Bispebjerg Bakke 23
DK-2400 Copenhagen NV
Denmark
Phone: +45 5330 9691
E-mail: misbah.noshela.ghazanfar@regionh.dk

Doi
10.23822/EurAnnACI.1764-1489.08

Summary
Omalizumab (anti-IgE) is used as add-on therapy for antihistamine refractory chronic urticaria patients. The most commonly reported adverse effects were headache, arthralgia, upper respiratory infections, fatigue, nausea and injection-site reactions. However, lately a few cases of hair loss have been reported. We describe a case of transient hair loss in a young female patient after initiating treatment with omalizumab. Despite this side effect, the patient continued with omalizumab treatment for 10 months with good effect.

Dear Editor,
We read with interest the recent case report by Konstantinou et al. (1) published in your journal about the use of omalizumab in three female patients with chronic spontaneous urticaria (CSU) who reported transient hair loss. In the case report by Konstantinou et al. (1) all three patients had previously been treated with non-sedating antihistamines, ranitidine and montelukast without any symptom relief. Furthermore, all of the patients were also treated with at least one short course of prednisolone due to severe angioedema and pruritus in the last four weeks before initiating treatment with omalizumab. Two of the female patients had no other known significant illnesses, whereas one of the patients was known with Hashimoto's thyroiditis since the past 12 months. All three patients reported transient hair loss after initiating treatment with omalizumab, however only one of the patients (with Hashimoto's thyroiditis) had visible alopecia areata. None of the patients discontinued their treatment.

Transient hair loss is not a commonly reported side effect of omalizumab. In the following we describe a similar case of a female patient from our dermatology department, who was treated with omalizumab and also experienced transient hair loss. The patient was a 27-year-old woman with a three week history of urticaria who was referred to our department in June 2015. At the time of the referral, she had abdominal pain and she reported a recent urinary tract infection, eye infection and orolabial herpes infection. Urine and routine blood tests were normal aside from CRP, which was elevated to 53 mg/l (normal < 10mg/l). The patient suffered from diffuse urticaria and swelling, primarily of her palms, soles and around her eyes. She was treated with non-sedating antihistamines four times daily as well as 50 mg prednisolone daily for three days with some symptomatic relief. However, a few days later her symptoms worsened and she was re-hospitalized. Routine blood tests showed elevation of CRP to 33 mg/l and leukocytes to 10.7 x 10^9/l (normal 3.5-8.8 x 10^9/l). Chest X-ray, throat culture and

urine sample and an abdominal ultrasound were all normal. A skin biopsy was also performed and it confirmed urticaria histologically with eosinophil as well as neutrophil infiltrates. The patient was treated with non-sedating antihistamines (fexofenadine 180 mg) four times daily and 50 mg prednisolone daily without relief. Montelukast 10 mg was added without any improvement. After three days with continuous aggravation of her urticaria rashes, omalizumab 300 mg was initiated with fast reduction in symptoms and she was discharged the following day. Prednisolone was tapered to 5 mg daily and due to recurrence of urticaria symptoms and development of concomitant joint pain and flu-like symptoms dapsone 100 mg daily was added and omalizumab was increased to 300 mg every second week, whereas antihistamines, montelukast and prednisolone were discontinued. After two weeks the patient was symptom free and she discontinued dapsone, whereas omalizumab was decreased to 300 mg every fourth week. After three months of treatment, the patient reported shedding about 1/3 of her scalp hair. There was no visible alopecia areata. The patient continued with omalizumab treatment and scored zero on the urticaria activity score in the past week (UAS7). After two months, the patient reported regrowth of her hair. The patient was completely symptom free; therefore the dosing interval of omalizumab was prolonged from four weeks to five weeks and then to six weeks. The patient discontinued treatment with omalizumab after 10 months as she was completely asymptomatic (UAS7 = 0). However, the patient experienced flare up shortly after and was retreated with 300 mg omalizumab every sixth weeks. Interestingly, the patient experienced no hair loss during this round of treatment or any other adverse effects.

We agree with Konstantinou et al. (1) that hair loss could be a transient side effect of omalizumab seen among some CSU patients. Particularly, since mast cells have been shown to be involved in the hair cycle (2), manipulation of mast cell activity could be speculated to account for this. However, the association with omalizumab is uncertain, as the patient described by Konstantinou et al. (1) with visible alopecia areata also had Hashimoto's thyroiditis, which is commonly associated with hair loss (3). Likewise, the hair loss in our case could have been caused by urticaria itself or the infectious diseases prior to urticaria. Also, our patient, and the cases described by Konstantinou et al. (1) were all treated with prednisolone, which has also been associated with hair loss. Furthermore, it is worth noting that hair loss is not a reported adverse effect in clinical phase trials of CSU and omalizumab. The most commonly reported adverse effects were headache, arthralgia, upper respiratory infections, fatigue, nausea and injection-site reactions (4-6). We conclude that hair loss may have multiple causes and that these must be accommodated in the explanation of the possible association between omalizumab use and hair loss in patients with urticaria.

References

1. Konstantinou G. N., Chioti A. G., Danilidis M. Self-reported hair loss in patients with chronic spontaneous urticaria treated with omalizumab: an under-reported, transient side effect? Eur Ann Allergy Clin Immunol. 2016;48:205-7.
2. Paus R., Peters E., Eichmiiller S et al. Neural Mechanisms of Hair Growth Control. JID Symposium Proceedings. 1997;2:61-8.
3. Thomas E. A., Kadyan R. S. Alopecia areata and autoimmunity: a clinical study. Indian J Dermatol. 2008;53:70-4.
4. Saini S., Bindslev-Jensen C., Maurer M. et al. Efficacy and safety of omalizumab in patients with chronic idiopathic / spontaneous urticaria who remain symptomatic on H_1 antihistamines: a randomized, placebo-controlled study. J Clin Investig Dermatol. 2015;135:67-75.
5. Saini S, Rosen K. E., Hsieh H.-J. et al. A randomized, placebo-controlled, dose-ranging study of single-dose omalizumab in patients with H1-antihistamine-refractory chronic idiopathic urticaria. J Allergy Clin Immunol Pract. 2011;128:567-73.
6. Maurer M, Altrichter S., Bieber T et al. Efficacy and safety of omalizumab in patients with chronic urticaria who exhibit IgE against thyroperoxidase. J Allergy Clin Immunol. 2011;128:202-9.

N. Syrigos[1], D. Grapsa[2], E. Syrigou[1]

Omalizumab for refractory chronic spontaneous urticaria during concurrent immunomodulatory therapy for multiple sclerosis

[1]Allergy Department, "Sotiria" General Hospital, Athens, Greece
[2]3rd Department of Medicine, "Sotiria" General Hospital, Medical School, University of Athens, Athens, Greece

Key words
angioedema; antihistamines; chronic spontaneous urticaria; omalizumab; multiple sclerosis.

Corresponding author
Dimitra Grapsa
3rd Department of Medicine, "Sotiria" General Hospital
University of Athens, School of Medicine, Athens, Greece
Mesogion 152, 115 27 Athens, Greece
Phone: +30 210 7751063
Fax: +30 210 7751063
E-mail: dimgrap@yahoo.gr

Doi
10.23822/EurAnnACI.1764-1489.20

Summary
Data derived from previous clinical trials and real-life studies have shown that omalizumab may represent an effective third-line treatment option for patients with chronic spontaneous urticaria (CSU) refractory to standard antihistamine treatment. Nevertheless, the safety and efficacy of omalizumab treatment for CSU, when administered concurrently with other immunomodulatory agents remains largely unknown. We herein present the case of a female patient with relapsing-remitting multiple sclerosis (RRMS), under treatment with interferon beta-1a, azathioprine and gabapentin, who was successfully treated with omalizumab for refractory CSU. To the best of our knowledge, this is the first reported case attesting to the safety and efficacy of omalizumab for CSU when administered concurrently with other immunomodulatory agents.

Omalizumab is a humanized anti-IgE monoclonal antibody currently approved by the Food and Drug Administration (FDA) for the treatment of moderate to severe allergic asthma and chronic spontaneous urticaria (CSU) not responding to standard antihistamine treatment. Off-label uses of omalizumab include an expanding list of various allergic and autoimmune conditions, while its exact mechanism of action, as well as its potential synergies or interactions with other drugs, remain relatively unknown (1).
To the best of our knowledge, there are no previous reports supporting the efficacy and safety of omalizumab when administered concurrently with other immunomodulatory agents. We herein present the clinical presentation, course and outcome of a patient with relapsing-remitting multiple sclerosis (RRMS), under immunomodulatory therapy, who was successfully treated with omalizumab for refractory CSU.

A 53-year old female, with a 7-year history of RRMS - treated with interferon beta-1a, azathioprine and gabapentin - presented to the Allergy Department of Sotiria Athens General Hospital, with widespread urticaria and angioedema lasting for 12 weeks. Her remaining medical history was remarkable for essential hypertension, treated with amlodipine / valsartan. Two weeks after appearance of urticaria, all drugs for MS and hypertension were discontinued, with no resolution of symptoms, and reinstated approximately four weeks later, again with no symptomatic change. Following administration of first-line antihistamine monotherapy (levocetirizine, 10 mg once daily) and repeated courses of corticosteroids (oral methylprednisolone, 64 mg/day and 6 intramuscular injections of hydrocortisone, 250 mg/injection), no significant symptomatic improvement was noted and the patient was referred by her primary care phy-

sician to an allergy clinic for further diagnostic evaluation and therapeutic management.

At the time of the patient's presentation to our department a comprehensive investigation for potential underlying causes of chronic urticaria was initiated. More specifically, a detailed clinical history, physical examination, chest X-ray and thorough laboratory testing, including complete blood count with differential, complete biochemical profile, erythrocyte sedimentation rate, C-reactive protein and thyroid-stimulating hormone levels (TSH), failed to reveal any clinically relevant findings; serological tests for hepatitis B surface antigen (HBsAg), human immunodeficiency virus (HIV), thyroid autoantibodies (anti-thyroid peroxidase and anti-thyroglobulin antibodies), antinuclear antibodies (ANA), anti-double stranded DNA (anti-dsDNA) antibodies and rheumatoid factor, were all negative. Serum immunoglobulins (IgG, IgA, IgM and IgE), complement C3 and C4 and rheumatoid factor levels were all within normal limits. Autologous serum skin test (ASST) was also performed, with negative results. Treatment was modified with switch from levocetirizin to cetirizine (20 mg x 4 daily), and subsequent addition of montelukast (10 mg once daily) and ranitidine (150 mg twice daily), with no significant improvement. An additional 9-day course of methylprednisolone (total dose of 188 mg) also failed to significantly improve symptoms. The patient was thereafter placed on omalizumab treatment (300 mg by subcutaneous injection every 4 weeks), with complete remission of symptoms within 2 days following administration of the first dose. At the time of her latest follow up visit, six months after initiation of omalizumab treatment, no recurrence of urticaria/angioedema, significant toxicity or alteration of her neurological status, as further confirmed by her attending neurologist, were noted.

CSU, otherwise known as chronic idiopathic urticaria (CIU), is a condition characterized by spontaneous appearance of itchy wheals (hives), angioedema, or both, that recur for a period longer than 6 weeks and have no identifiable external trigger (2,3). Data derived from phase III clinical trials have demonstrated that omalizumab may represent a safe and effective third-line treatment option in CSU (4-6). Nevertheless, the short- and long-term safety and efficacy of this drug in CSU patients with comorbidities treated with other biologic response modifiers remains essentially unexplored.

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous disease, with RRMS as its most common form. Treatment for RRMS is primarily aimed at reducing the frequency of relapses by suppressing or modulating the immune system response (7). Various immunomodulatory and immunosuppressive agents are now available for RRMS, including, among others, injectable drugs with well-established safety and efficacy, such as interferon beta-1a, azathioprine and glatiramer acetate, as well as new therapies such as fingolimod, the first oral drug approved for the treatment of MS, or monoclonal antibodies, such as natalizumab, carrying the potential of higher efficacy but, also, an increased risk of serious complications (7).

Our reported case attests to the short-term efficacy and safety of omalizumab treatment for CSU when administered concurrently with interferon beta-1a and/or azathioprine. Additional post-marketing safety data regarding concomitant use of omalizumab with other immunomodulatory agents are warranted.

Summary statement

The safety and efficacy of omalizumab treatment for refractory CSU in patients receiving other immumodulatory agents has not been previously evaluated. We herein report the case of a patient with refractory CSU successfully treated with omalizumab, while receiving concurrent immunomodulatory treatment for RRMS.

References

1. El-Qutob C. Off-label uses of omalizumab. Clin Rev Allergy Immunol. 2016;50:84-96.
2. Zuberbier T, Aberer W, Asero R et al. The EAACI/GA(2)LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria: the 2013 revision and update. Allergy. 2014;69:868-87.
3. Bernstein JA, Lang DM, Khan DA et al. The diagnosis and management of acute and chronic urticaria: 2014 update. Journal of Allergy and Clinical Immunology. 2014;133:1270-7.
4. Kaplan A, Ledford D, Ashby M et al. Omalizumab in patients with symptomatic chronic idiopathic/spontaneous urticaria despite standard combination therapy. Journal of Allergy and Clinical Immunology. 2013;132:101-9.
5. Maurer M, Rosen K, Hsieh HJ et al. Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. New England Journal of Medicine. 2013;368:924-35.
6. Saini SS, Bindslev-Jensen C, Maurer M et al. Efficacy and safety of omalizumab in patients with chronic idiopathic / spontaneous urticaria who remain symptomatic on H1 antihistamines: a randomized, placebo-controlled study. Journal of Investigative Dermatology. 2015;135:925.
7. Cross AH, Naismith RT. Established and novel disease-modifying treatments in multiple sclerosis. Journal of Internal Medicine. 2014;275:350-63.

European Annals
of Allergy and
Clinical Immunology

The online submission system

European Annals of Allergy and Clinical Immunology uses an online submission and review system for all papers evaluation.
Electronic submission allows a more efficient processing of manuscripts and offers Authors the option to track the progress of the review process whenever they need to.
The link to the editorial system is http://eaaci.edmgr.com, it is also available on the Journal website: *www.eurannallergyimm.com.*

The Authors are invited to submit their manuscripts through the online editorial system; manuscripts sent by e-mail, post or fax are not considered for publication.
All the Authors should read carefully the Guide for Authors before starting their submissions. Full information about the manuscript preparation are available on the Journal website.
During submission, Authors will be first asked to select the article type, enter the manuscript title and provide Author information. Through a menu, a general topic area should be selected: these will help to match manuscripts to the best available editors and reviewers.
Reviewers will access papers via the editorial system platform and will be invited and sent to it by email.

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