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Table of Contents

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Milk hypersensitivities: where is the grey line regarding their dietary management?

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Key words
cow's milk allergy; cow's milk intolerance; dietary management; calcium supplementation; vitamin D

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Summary
The proportion of people suffering or reporting to have a hypersensitivity caused by cow's milk consumption is increasing, and even health professionals often face difficulties into elaborating properly with a milk reaction due to misdiagnosis. The scope of this review is to present literature data that lead into putting the border line between cow's milk allergy and cow's milk intolerance, mainly focusing on how the different pathophysiology leads to their different dietary diagnosis and management.

Introduction

According to the European Academy of Allergy and Clinical Immunology (1) any adverse reaction to food is called food hypersensitivity. Non-toxic adverse reactions to foods are divided according to the implication or not of the immune system into food allergies and food intolerances. Apart from these, reactions to toxic substances and psychological reactions also belong into the adverse reactions to foods.

Despite the clear differentiation of their pathophysiological mechanisms, food allergies and intolerances very often confuse people thinking themselves as sufferers of food hypersensitivity, without being able to confirm this by proper diagnostic examination, food exclusion and food challenges or reintroduction of the offending food. Additionally, in the clinical practice often food allergy and intolerance are misdiagnosed, due to the time delay between ingestion and symptoms and insufficient diagnostic tools. On the other hand, food allergies and intolerances, when not diagnosed and managed properly, can affect growth or nutritional status significantly, in some cases can be life threatening, but also can reduce significantly the quality of life of the sufferers (2).

Overall, milk hypersensitivities are common, with milk being the major trigger of allergic reactions in childhood (2-3%) (3,4), but also lactose intolerance affecting a high proportion of adults, reaching the incredible number of 80-95% the UK and Germany (5).

This review aims to put the borderline of different types of milk hypersensitivities in order to ensure the appropriate dietary management further to proper medical diagnosis and treatment.

Materials and methods

A literature search was performed on PubMed, ScienceDirect, Springerlink, The Cochrane Library. Articles with evidence and recommendations regarding the phenotype (characteristics) and

dietary management of specific on cow's milk related hypersensitivities published up to December 2014 were collected.
Search terms included "cow's milk allergy", "lactose intolerance", "IgE-mediated", "non-IgE mediated", "Dietary management", "Growth", "Elimination diet", "Calcium", "Vitamin D".

Cow's milk allergy (CMA)

Following the general terminology of food allergy, CMA is any reaction caused after milk consumption that triggers the immune system. The main cow's allergens are casein (αs1-, αs2-, β-, and κ-casein) and whey homologs (α-lactalbumin, β-lactaglobulin). Three types of CMA present: IgE-mediated, non-IgE/cell-mediated and the mixed form (IgE and non-IgE) (3).

Epidemiology

Although reports for milk allergy are high, ranging from 1-17.5% among preschoolers (6), the actual diagnosed incidences are lower ranging to 2-4% in infancy (7-9). Symptoms develop usually during infancy, within the first month after cow's milk protein introduction in the diet, whereas remission of symptoms develops at 3 years of age in a rate of 85-90% of children (10). Data for milk allergy in adulthood indicate low prevalence ranging from 0.1-0.5% of the population (11).

Immediate reactions

Specific-IgE antibodies are produced against milk allergens after exposure to CM, at any age but mainly during infancy - even in breastfed infants- and early childhood (12). Symptoms occur from minutes up to 2 hours after ingestion and involve one or more systems, with symptoms from the skin (urticaria and angioedema), gastrointestinal (nausea, vomiting and diarrhea), nervous, cardiac (13) and respiratory system (rhinoconjunctivitis and asthma), delayed growth and failure to thrive, but also anaphylaxis (14-16).

Non-IgE mediated CMA

Apart from the cases of the IgE-mediated features of immediate hypersensitivity, there is an equal proportion of pediatric patients presenting symptoms mediated from non-IgE mechanisms with symptoms of atopic eczema, gastro-oesophageal reflux, persistent crying, diarrhea and sometimes constipation. Diagnosing non-IgE mediated CMA can be challenging as those symptoms are common in infancy, even in the absence of atopy. Removing milk from the diet, follow up of symptoms resolution and re-challenge can lead to a clear diagnosis.
Non-IgE mediated CMA describes unclear mechanisms of T-cells (probably Th2) responses, without the production of IgE antibodies, but mediated by proinflammatory cytokines (17), occurring usually in 1-3 hours up to 2-3 days after milk ingestion (18). Symptoms from the gastrointestinal (GI) tract and the skin are the most common manifestations. CM-protein induced enterocolitis syndrome (CMPIES) involves the whole GI (19,20) with severe symptoms of repetitive vomiting usually 2-4 hours after ingestion and/or diarrhea and lethargy 5-10 hours after consumption (21), lack of other symptoms related to the offending food, and a resolution of symptoms after its removal from the diet (22,23). In infants with FPIES caused by cow's milk, breastfeeding is recommended, although there are few reports of infants with chronic symptoms of regurgitation, colic, diarrhea and failure to thrive, caused from CM proteins passing through breast milk (24). Interestingly, it is also reported a newborn with CMPIES before first feeding, with persistent symptoms when fed with CM formula and symptoms resolved when the last was discontinued and initially with intravenous nutrition and then with a diet of extensively hydrolyzed formula with breast milk (25). Avoidance of milk and its products from the nursing mother and casein-hydrolyzed formulas or amino acid formulas led to FPIES symptoms resolution (19,26).
CM-induced enteropathy involves the small bowel and CM induced proctitis and proctocolitis of the rectum and colon, with remission of symptoms when milk is removed from the diet (3,18).

Diagnosis of CMA

Following the general diagnostic approach of food allergy, for diagnosing CMA detailed medical and dietary history should be taken, followed by physical examination, SPTs, sIgE measurements, elimination diet for milk and milk products and oral food challenges (27)

Dietary management

Elimination diet has still the key role into managing CMA (28,29). Heat or enzymatic treatment results to formulas at a variable range of hydrolysis of cow's milk proteins, and together with elemental (amino acid) formulas are the forefront alternative choices to CM (30,31). Soy milk after the 6th month of age is an option (32), but hydrolyzed rice-based formula is under consideration as its nutrient adequacy still needs to be further studied as up to date results are controversial (33,34). Probiotics role is investigated in various aspects: in prevention (35-37) or reduction of atopy (38,39), controlling eczema (37,40,41) increasing the proportion of acquired tolerance in milk allergic children (28), but evidence is still being sought (42-44). Notwithstanding some hypoallergenic, hydrolyzed or amino-acid infant formulas are supplemented with probiotics, although scientific research is not yet clear regarding their effectiveness. **Table 1** is a comparative presentation of milk formulas with different grade of hydrolyzation (partially, extensively, elemental), or derived from different sources (cow's milk, soya, rice) regard-

ing their macronutrient and selected important micronutrient composition (calcium, iron, zinc).
Donkey's, mare's, camel's and even pig's milk (45-49) have also been proposed as safe alternatives for some CMA patients, but need to be further evaluated in terms of nutrients adequacy and cross reactivity to CM proteins (15). Contradictory, goat's and sheep's milk frequently cause reactions due to the high sequence homology between these related species (50-52).

The selection of the correct formula is based on the patient's history and clinical evaluation after introduction of the new CM substitute. Allergic symptoms, stool patterns, regurgitation, or even frequency of crying are some of the indicators of acceptability (34,35). Educating patients and guardians into avoiding all possible sources of milk is essential in order to ensure accidental reactions. Although according to EU Regulation 1169/2011 (53) milk labeling on commercial products is mandatory, still dif-

Table 1 *- Comparison of milk formulas with different grade of hydrolyzation (partially, extensively, elemental), lactose content or derived from different sources (cow, soya, rice).*

Category	Product Name	Brand Name	Energy	Carbohydrates	Fat	Protein	Calcium	Iron	Zinc
Infant Formula	S-26 Original Newborn	Pfizer	67.1	7.2	3.6	1.5	46	0.8	0.6
Infant Formula	Enfalac A+	Mead Johnson	66	6.9	3.5	1.65	44	0.79	1
PARTIALLY HYDROLYZED FORMULAS: the high content of high Molecular Weight Peptides (MW > 4.000 Dalton and 5% peptides with MW > 15.000) and unaffected protein molecules explains their intact allergenic activity Indications: allergy prevention when positive history of atopy.									
HA	NAN HA Gold	Nestle	67	7.8	3.4	1.3	49	0.7	0.7
	Similac Advance HA	Abbot	64.3	6.92	3.62	1.33	52.7	1.22	0.51
	Aptamil Gold HA	Danone Nutricia	65	7.2	3.4	1.5	46	0.53	0.5
EXTENSIVELLY HYDROLYSED FORMULAS (EHF): extensive hydrolysis results high quantity of small peptides (di- and tripeptides. MW < 1200 Dalton) and smaller quantities of large peptides and free amino acids. This change of the protein structure. reduces the antigenicity and allergenicity of 10 to 100 times compared with conventional milk. The possibility of raising a reaction even in milks with extensive hydrolysis is due to the fact that the remaining epitopes can be recognized by the immune system of very sensitive infants Indications: Milk and soy allergy. Eosinophilic Enterocolitis. Eosinophilic Oesophagitis. Eosinophilic Gastroenteritis.									
	Nutramigen with Enflora	Enfamil	70.4	7.25	3.73	1.97	66.2	1.27	0.7
	Alfare	Nestle	70	7.7	3.6	2.1	54	0.7	0.7
	Pregomin Pepti	Danone	66	6.8	3.5	1.6	50	0.8	n/a
ELEMENTAL (AMINO ACID) FORMULAS: manufactured from free amino acids Indications: CMA when EHF cannot be tolerated.									
Amino Acid	Elecare	Abbot	70.4	7.54	3.38	2.18	81.7	1.27	0.81
	Neocate	Nutricia	67	7.84	3.02	2.08	83.1	1.24	1.11
	Nutramigen AA	Mead Johnson		7	3.6	1.86	64	1.22	0.68
LACTOSE FREE FORMULAS Indications: Lactose intolerance.									
Lactose-free	S-26 Lactose Free	Pfizer	67.1	7.2	3.6	1.5	55mg	0.8	0.6
	Lactose Free	SMA	67	7.2	3.6	1.5			
Alternative products									
Soy milk	Prosobee	Enfamil	70	10.6	5.3	2.5	73.9 mg	1.27	0.85
Partially hydrolyzed rice milk	Novarice	Novalac	67.9	7.4	1.8	3.4	60.8	0.9	0.7

Table 2 *- Terminology used for labeling CM on commercial food products.*

Terms used for milk labeling: Whey, Rennet, Casein, Cheese, Lactalbumin, Curd, Quark, Yogurt
Terms that might imply the presence of milk protein: Butter, Milk fat, Praline, Sherbet, Ghee

ferent terms can be found on food labels of products produces and marketed. **Table 2** presents some common terms used for labeling milk or implying the possible presence of milk.

Processed products, baked/ cooked products

Although the effect of industrial processing (pasteurization, ultra-high-temperature heating, or dry blending for cow's milk formula) remains controversial to whether it can affect the antigenic / allergenic properties of cow's milk proteins (54-57), 70% of the children with diagnosed CMA can tolerate baked products, probably due to the change of the isoforms resulting from the prolonged heating in higher temperatures (58-61). This also applies to some patients with CM eosinophil esophagitis (62). Accordingly, baked milk is used for oral immunotherapy protocols (63,64) successfully, as it probably accelerates tolerance (65). In a big rate of patients, being able to introduce baked milk gradually into their diet is extremely significant as it improves tolerance and improves significantly quality of life (66).

Growth and nutritional assessment

Food allergies result in malnourished children, according to several studies (67-69), making normal growth one of the major concerns also to CMA allergic children (4) (70). Recently, Harvey et al. presented that an amino acid based formula containing synbiotics could ensure normal growth in healthy, non-allergic children exclusively fed with this formula (71). Similarly, earlier publications for various extensively hydrolyzed and elemental formulas with docosahexaenoic acid (DHA) and arachidonic acid (ARA) have shown that these products sustain growth in healthy or CM-allergic infants and are well tolerated from the last (72). Contradictorily, many publications emphasize the link between milk allergy and decreased growth in children (71,72) when they do not consume another appropriate substitute, as they are found shorter and to weigh less when compared with their matched counterparts (72).
Therefore, appropriate nutritional assessment, analysis and management are essential to avoid growth impairment in this population. A nutrition-focused medical history and nutrition-focused physical examination can place the link between nutrient adequacy as denoted from the diet history and growth (75,76). These will evaluate anthropometrics (weight, height, BMI, head circumference) measurements compared with appropriate local growth charts, biochemical evaluation also taking into account amylase, iron, calcium, vitamin D levels, as well as medical history, diet history including dietary intake and evaluation of children's and family's eating practices and environmental factors such as activity level of the patient and ability to socialize due to the CM.
Based on the above, nutritional diagnosis will lead to the appropriate dietary guidelines, that will ensure nutrient adequacy especially for protein, calcium and vitamin D (77,78), but also facilitate and protect quality of life. Evidenced-based alternatives should be provided to the family, together with detailed explanation on the reasons for introducing to the child's diet the "new" foods. **Table 3** and **table 4** present good sources of Calcium and vitamin D respectively, in comparison to CM and various CM-products.

CM allergy in older children and adults

Although CM allergy is more common during infancy and early childhood, when this does not resolve or when it occurs in adulthood, then symptoms are severe and often anaphylactic, affecting enormously the patients' quality of life (79,81).

Lactose intolerance

Lactase is responsible for hydrolyzing lactose into its components: monosaccharides, glucose and galactose. Lactose intolerance (LI) is caused due to a downregulation of lactase expression in the small intestine and can explain symptoms of bloating, flatulence, diarrhea (81).
Congenital lactase deficiency, also called congenital alactasia, occurs in infancy due to mutations in the LCT gene, which is responsible for the lactase synthesis. Unbroken lactose from breast milk or formula causes severe diarrhea leading to dehydration and weight loss, if milk is not substituted with a lactose-free formula.
In adulthood, lactose intolerance is caused by gradually decreasing activity (expression) of the LCT gene after infancy. LCT gene expression is controlled by regulatory element of the DNA located within a nearby gene (MCM6). Some individuals have inherited changes in this element that lead to sustained lactase production in the small intestine and the ability to digest lactose throughout life. People without these changes have a reduced ability to digest lactose as they get older, resulting in the signs and symptoms of lactose intolerance.
The severity of the symptoms depends on the amount of the lactase produced, but also the amount of lactose consumed from the diet, and the type of meal, the colonic macrobiota and individual sensitivity and perceptions. The last lays to over-self-diagnosis as lactose intolerant, when this is not confirmed with genetic analysis, H2-breath test or duodenal biopsies for measuring lactase expression. Blinded lactose challenges are also under investigation as a diagnostic tool for LI (81). Although the prevalence of LI is difficult

Table 3 *- Dairy (shaded) and Non-dairy Calcium-rich foods (non-shaded).*

Food, Standard Amount	Calcium (mg)
Plain yogurt, non-fat (13 g protein / 8 oz), 8-oz container	452
Romano cheese, 1.5 oz	452
Pasteurized process Swiss cheese, 2 oz	438
Plain yogurt, low-fat (12 g protein / 8 oz), 8-oz container	415
Fruit yogurt, low-fat (10 g protein / 8 oz), 8-oz container	345
Swiss cheese, 1.5 oz	336
Ricotta cheese, part skim, ½ cup	335
Pasteurized process American cheese food, 2 oz	323
Provolone cheese, 1.5 oz	321
Mozzarella cheese, part-skim, 1.5 oz	311
Cheddar cheese, 1.5 oz	307
Fat-free (skim) milk, 1 cup	306
Muenster cheese, 1.5 oz	305
1% low-fat milk, 1 cup	290
Low-fat chocolate milk (1%), 1 cup	288
2% reduced fat milk, 1 cup	285
Reduced fat chocolate milk (2%), 1 cup	285
Buttermilk, low-fat, 1 cup	284
Chocolate milk, 1 cup	280
Whole milk, 1 cup	276
Yogurt, plain, whole milk (8 g protein / 8 oz), 8-oz container	275
Ricotta cheese, whole milk, ½ cup	255
Blue cheese, 1.5 oz	225
Mozzarella cheese, whole milk, 1.5 oz	215
Feta cheese, 1.5 oz	210
Fortified ready-to-eat cereals (various), 1 oz	236-1043
Soy beverage, calcium fortified, 1 cup	368
Sardines, Atlantic, in oil, drained, 3 oz	325
Tofu, firm, ½ cup	253
Pink salmon, canned, with bone, 3 oz	181
Collards, cooked from frozen, ½ cup	178
Molasses, blackstrap, 1 Tbsp	172
Spinach, cooked from frozen, ½ cup	146
Soybeans, green, cooked, ½ cup	130
Turnip greens, cooked from frozen, ½ cup	124
Ocean perch, Atlantic, cooked, 3 oz	116
Oatmeal, plain and flavored, instant, fortified, 1 packet prepared	99-110
Cowpeas, cooked, ½ cup	106
White beans, canned, ½ cup	96
Kale, cooked from frozen, ½ cup	90
Okra, cooked from frozen, ½ cup	88
Soybeans, mature, cooked, ½ cup	88
Blue crab, canned, 3 oz	86
Beet greens, cooked from fresh, ½ cup	82
Pak-choi, Chinese cabbage, cooked from fresh, ½ cup	79
Clams, canned, 3 oz	78
Dandelion greens, cooked from fresh, ½ cup	74
Rainbow trout, farmed, cooked, 3 oz	73

Table 4 *- Vitamin D Food sources (82, 97).*

Food	IUs per serving*	Percent DV**
Cod liver oil, 1 tablespoon	1,360	340
Swordfish, cooked, 3 ounces	566	142
Salmon (sockeye), cooked, 3 ounces	447	112
Tuna fish, canned in water, drained, 3 ounces	154	39
Orange juice fortified with vitamin D, 1 cup (check product labels, as amount of added vitamin D varies)	137	34
Milk, nonfat, reduced fat, and whole, vitamin D-fortified, 1 cup	115-124	29-31
Yogurt, fortified with 20% of the DV for vitamin D, 6 ounces (more heavily fortified yogurts provide more of the DV)	80	20
Margarine, fortified, 1 tablespoon	60	15
Sardines, canned in oil, drained, 2 sardines	46	12
Liver, beef, cooked, 3 ounces	42	11
Egg, 1 large (vitamin D is found in yolk)	41	10
Ready-to-eat cereal, fortified with 10% of the DV for vitamin D, 0.75-1 cup (more heavily fortified cereals might provide more of the DV)	40	10
Cheese, Swiss, 1 ounce	6	2

*IUs = International Units. ** DV = Daily Value.

to discern and varies among different populations, it is considered to affect 30% of the population, but its frequency varies considerably between different ethnic groups and population. The lowest rates are seen in white North Europeans, North Americans and Australasians from 4.7% in British populations to 17% in Finland and Northern France. The highest rates tend to be found in South America, Africa and Asia with approximately 50% of the population affected and almost 100% in some Asian countries. Ethnic groups also tend to lose lactase activity differently, with Chinese and Japanese lacking 80-90% of lactase activity within 3-4 years after weaning, Jews and Asians losing 60-70% over several years post weaning and white Northern Europeans may take up to 18-20 years for lactase activity to reach its minimal expression (5).

Diagnosis of LI

Several methods have been proposed for LI diagnosis, such as genotype determination, Lactose Tolerance Test, Quick Lactose Test. Nevertheless, the most reliable, inexpensive and non-invasive test is Lactose Breath Test, which has shown excellent specificity and good sensitivity (82).

Dietary management of LI

Calcium inadequacy is the main nutritional risk for lactose-intolerant patients. Interestingly, most lactose intolerant patients can tolerate without a problem up to 12 grams of lactose, equal to 1 cup of milk, with minor symptoms, especially if these are consumed with other foods or spread over the day. Some studies have examined whether it is possible to induce adaptation by consuming incremental lactose loads over a period of time, but the evidence in support of this strategy is inconsistent (83). Alternatively, low-lactose dairy products, including yogurt, aged cheeses (such as Cheddar and Swiss) or lactose-reduced or lactose-free milk are good sources of calcium without provoking symptoms. Additionally, nondairy food sources high in calcium should be included in the diet of all milk allergic and intolerant individuals, such as teleost fish such as anchovy, small sardines and mola (84) that can be consumed with the bone, chicken bone cartilage, kale, bok choy, Chinese cabbage, broccoli, collards, but also fortified with calcium foods such as juices and cereals (85,86). Calcium bioavailability should be considered when selecting plant sources as this might vary significantly, and from some is not that well absorbed as from others (87) (**table 3**).

Calcium supplementation in milk hypersensitivities

Milk is the first food for neonates and infants ensuring proper development, by providing the necessary nutrients and energy. Furthermore, it has a crucial role in the formation of the bone mass. Especially children with CMA, but also patients with lac-

tose intolerance are under a high risk of inadequate quantities of calcium in the diet resulting in reduced bone mass density and early osteoporosis, due to disturbances in bone mineralization and metabolism (79). A milk-free diet is also related to fractures during growth (88-90). Nevertheless, symptoms resolve in the incidence of milk desensitization or with appropriate supplementation or substitution of essential minerals (90-94).

Adequate calcium intake is only ensured when these patients, while being on a nondairy diet, have the appropriate nutritional supervision and guidance (79).

Supplementation with calcium and vitamin D can be used in order to prevent nutritional rickets. But recommended dietary allowance and tolerable upper intake levels should be considered in order to provide adequate amounts and avoid adverse / toxic reaction (**tables 5** and **6**).

There are several available forms of calcium in supplements, with two most extensively used: carbonate and citrate. Calcium citrate was at first suggested to be easier absorbed, even in empty stomach and also useful for people suffering from achlorydria, inflammatory bowel disease or absorption disorders (97), but these results were not later confirmed by other studies (98). The amount of calcium absorbed depends on the total amount of elemental calcium consumed at one time, with the bioavailability and solubilization playing an important role under conditions of low calcium intake (≤ 500 mg), but with this becoming insignificant in high calcium doses (> 800 mg) (99-101).

Table 5 *- Calcium and vitamin D recommended dietary allowance according the age group (98)*

Age	Calcium Recommended Dietary Allowance (mg/day)	Vitamin D Recommended Dietary Allowance (IU/day)
Infants 0 to 6 months	*	**
Infants 6 to 12 months	*	**
1 - 3 years old	700	**
4 - 8 years old	1,000	600
9 - 13 years old	1,300	600
14 - 18 years old	1,300	600
19 - 30 years old	1,000	600
31 - 50 years old	1,000	600
51 - 70 years old	1,000	600
51 - 70 year old females	1,200	600
71+ years old	1,200	800
14 - 18 years old, pregnant/lactating	1,300	600
19 - 50 years old, pregnant/lactating	1,000	600

*For infants, adequate intake is 200 mg/day for 0 to 6 months of age and 260 mg/day for 6 to 12 months of age.
**For infants, adequate intake is 400 IU/day for 0 to 6 months of age and 400 IU/day for 6 to 12 months of age

Table 6 *- Tolerable Upper Intake Levels (ULs) a. for Calcium (average nutrient intake unlike to pose adverse reactions) b. for Vitamin D.*

	Calcium			
Age	**Male**	**Female**	**Pregnant**	**Lactating**
0-6 months	1,000 mg	1,000 mg		
7-12 months	1,500 mg	1,500 mg		
1-8 years	2,500 mg	2,500 mg		
9-18 years	3,000 mg	3,000 mg	3,000 mg	3,000 mg
19-50 years	2,500 mg	2,500 mg	2,500 mg	2,500 mg
51+ years	2,000 mg	2,000 mg		
Vitamin D				
0-6 months	1,000 IU (25 mcg)	1,000 IU (25 mcg)		
7-12 months	1,500 IU (38 mcg)	1,500 IU (38 mcg)		
1-3 years	2,500 IU (63 mcg)	2,500 IU (63 mcg)		
4-8 years	3,000 IU (75 mcg)	3,000 IU (75 mcg)		
≥ 9 years	4,000 IU (100 mcg)	4,000 IU (100 mcg)	4,000 IU (100 mcg)	4,000 IU (100 mcg)

Conclusion

Cow's milk allergy is less common than lactose intolerance, affecting 0.6% to 0.9% of the population. Nevertheless, cow's milk allergic individuals require strict avoidance of cow's milk proteins containing products, as severe life threatening reactions may be elicited and are therefore at higher risk of obtaining insufficient protein and calcium intake. Avoidance of cross-reactive food products should be considered when providing guidance regarding their dietary management. In lactose intolerance, which is much more frequent especially in late puberty and adulthood, symptoms are mild and lactose free products together with alternative non-dairy products can reduce the risk of calcium inadequacies. For all cow's milk hypersensitive patients,

calcium and vitamin D supplementation should be considered at individual's basis when diet is not considered adequate in order to protect bones mass density.

Overall a nutrition focused medical and physical examination should be obtained by experienced dietitians and appropriate counseling should be provided in order to reduce the risk of growth or nutrients impairments.

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Food allergy: practical approach on education and accidental exposure prevention

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Key words
food allergy; accidental exposure; allergen avoidance; food labelling; cross-contact

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Summary
Food allergies are a growing problem and currently the primary treatment of food allergy is avoidance of culprit foods. However, given the lack of information and education and also the ubiquitous nature of allergens, accidental exposures to food allergens are not uncommon. The fear of potential fatal reactions and the need of a proper avoidance leads in most of the cases to the limitation of leisure and social activities. This review aims to be a practical approach on education and accidental exposure prevention regarding activities like shopping, eating out, and travelling.
The recommendations are focused especially on proper reading of food labels and the management of the disease, namely in restaurants and airplanes, concerning cross-contact and communication with other stakeholders.
The implementation of effective tools is essential to manage food allergy outside home, avoid serious allergic reactions and minimize the disease's impact on individuals' quality of life.

Introduction

A food allergy is defined as a reproducible and specific immune response that occurs on exposure to a given food, leading to adverse health effects (1). In most of the cases, this response is mainly IgE mediated (2). The signs and symptoms of food allergic reactions may be mucocutaneous (like eczema, hives or edema of glottis and tongue), gastrointestinal (like diarrhea, vomiting or abdominal pain), respiratory (like wheezing or shortness of breath) and cardiovascular (like low blood pressure and loss of consciousness) (1). In food allergic patients, anaphylaxis, an acute, severe and systemic reaction, can be potentially life-threatening if not appropriately treated (3).
Although the prevalence of food allergy is not well known, recent studies show that approximately 5% of adults and 8% of children have food allergies (4). Foods are one of the leading identifiable causes of anaphylactic reactions (5,6) and the first one in the pediatric age in Portugal (7-9). The most common food allergies are to cow's milk, egg, peanuts and nuts, fish, shellfish, wheat and soy, and these foods account for 90% of food allergic reactions (10). Currently, the primary treatment of food allergy is avoidance of the involved foods (1,11-14). Additionally, in case of anaphylaxis, the first-line treatment is intramuscular injection of epinephrine (3).
Lack of information and education, and also the ubiquitous nature of some allergens (like milk or egg) (15,16), can contribute to accidental dietary exposures to be not uncommon (17,18). Education is particularly needed in terms of food labels' reading given that misinterpretation of food labels is a common cause of accidental ingestion (11,19,20). On the other hand is pivotal that food industry makes an effort directed toward the complete, accurate and unambiguous labelling of food (19,20). Proper labelling and reading are both crucial to the success of avoidance diets (11,20,21).

A Portuguese study with 69 children with food allergy was conducted to identify the frequency and to characterize accidental food exposures. The results have shown that 68.1% failures in the eviction diet occur with accidental exposure, and about one third of them (36.8%) occurred at home (22). Accordingly to other studies, parents have reported that children reactions generally occur in familiar locations such as home or school (5,23). Nevertheless, a significant number of reactions begins to occur in places like restaurants and other catering establishments (5,13,24), particularly in the case of adults (23). This fact points for an important role of the *world outside home* in food allergy patient's daily life.

From this perspective, literature suggests that anaphylactic reactions are more common in adolescents and young adults probably because they start to take responsibility for making their food choices preferably outside the home (25-28).

Another important issue regarding food allergy is the heavy emotional burden that is brought to these patients (25,29-32). The fear of reactions and the need of a proper avoidance leads in most of the cases to the limitation of leisure and social activities with a wide impact on quality of life (5,22,30,33,34). For example, a considerable percentage of food allergic individuals that have suffered an allergic reaction in a restaurant generally decided to avoid dining out (35). Accordingly, food allergic individuals also limit their vacations given that traveling abroad presents a potential risk (36). Approximately 30% of passengers that have an in-flight reaction reported they no longer fly and 40% decided do not eat any food served on board (37). Thus, commitment and education of the patients, their families and also third parts which provide food is crucial to live safer in community and minimize the impact of the disease (12,14,28). In a study of *Worth et al.*, eating out, travelling and food labelling were the areas where food allergy patients considered needing more information (32). Therefore, this review aims to be a practical approach on education and accidental exposure prevention, inside and outside the home (shopping, eating out, and travelling).

Labeling and shopping

Shopping is the first barrier for food-allergy individuals. When food allergic patients go shopping, they should know that it is crucial to spend time to read properly the labels of every food product. A study with nut-allergic individuals has reported that these consumers spend 39% more time identifying proper foods than other consumers (38).

A considerable proportion of accidental exposures are attributed to inappropriate labeling, failure to read labels, and ignoring precautionary statements (39). This issue is particularly alarming if we regard that checking food labelling is one of the most used strategies by patients for food allergy management (32). Then food allergic individuals depend on clear and consistent labeling of food allergens (38-40) and also on proper education about how to read labels to improve confidence and compliance (1,12,34,39).

At present, efforts have been made in terms of food allergen labelling legislation. Current EU legislation requires the clearly declaration of any of the 14 regulatory allergens (cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk and products thereof, nuts, celery, mustard, sesame seeds, sulphur dioxide and sulphites, lupine, and molluscs) when used as ingredient of prepacked foods (41,42).

However, food allergic individuals should note that this legislation is effective only in the European Union, and therefore products bought in other countries could be covered by different legal labelling (43). Another important issue is that allergens may be described in numerous different ways on the food product labels (34,44). This is particularly relevant if we take in account that studies have reported that food allergic consumers are unable to correctly identify and recognize products which contained food allergens (45,46).

Thereby, for example, label ingredients that an individual should be aware in case of a milk allergy include casein, whey, ghee, curd, lactalbumin, lactoglobulin, lactulose, lactose (12,34). Consumers should also note that lactose free products could contain milk protein (47) and that milk from others mammalians than cow is not suitable too (11). In case of egg, it may be described as albumin, emulsifier, livetin, ovomucoid, ovalbumin, lysozyme or avidin (34). Natural flavors are another concerning question as they could refer to peanuts, tree nuts, milk, or any other food (12). Although food allergen labelling laws could be a great contribute to make food choices easier, they still do not regulate other issues such as the potential presence of hidden allergens due to cross-contact (48). A substance is a hidden allergen when it is unrecognized or not declared on the product ingredient label (49).

The labelling that concerns the potential presence of unintentional ingredients, for example due to cross-contact in processing lines, is generally described as precautionary allergen labelling (PAL), for example "may contain", and it is applied voluntarily by the food industry (11,43,48,50,51). This statements should only be applied if it is considered that there is an actual risk of allergen cross-contact thorough a risk management plan (20,43,52). The requirement to manage potential contamination regarding the protection of food allergic consumers is covered through European Commission Regulations 178/2002 and 852/2004, despite current legislation does not cover the PAL's use (43).

The widespread use of this PAL is frequently reported, and it is known to limit the choices for food allergic individuals and to lead these individuals to sometimes choose to miss the PAL

(21,40,43,51,53). Indeed, some food allergic consumers suspected that PAL is used merely to avoid litigation and that, given the high prevalence of these labels, total avoidance is almost impossible (26,43,54,55). Concerning that cross-contact is unpredictable and that threshold of clinical reactivity can vary among individuals, misunderstanding about PAL lead to risk-taking (13,14,43) and has been found to contribute to deaths from anaphylaxis (54). Yet, some studies have reported that some of products with PAL actually have traces of the cited allergen, so, and given this risk, the avoidance of products with PAL should be recommended for the consumer with food allergy (1,56-57).
Although PAL was introduced to ensure the safety of the consumers with food allergy, currently this labeling seems to not fulfill patients' needs. Regarding this, in addition to the patient responsibility, through the avoidance of the products with PAL, there should be a stronger commitment from the food industry in order to reestablish PAL credibility. The proper education of the stakeholders in production chain and also the standardization of the industrial processes, including risk assessment and communication could be significant tools. Additionally, the support of the governmental authorities in the process as well as the creation of legislation that covers the use of PAL could also be important.
Regarding other practical aspects of buying products, food allergic consumers must pay attention to prepackaged meals, juices and alcoholic beverages (may contain milk, nuts) and also nonfood products (like pet food, dental products, cosmetics and non-prescription medicines) (34). Allergens may also have unexpected sources. For instance, milk may be an ingredient of candies, ice-creams, chocolates, ham, sausages and processed meats, canned tuna fish, cereals, crackers and biscuits (34). Egg may be found in candies, pastry, sauces baked goods, meatballs, breaded meats and commercial egg substitutes (15,34).
Another misconception is that patients do not need to read a label each time they bought a product (58), especially if the consumption is common. Food industry may alter the products' formulation without advice, so it is very important that patients read ingredient labels every time they purchase a product (34,48).
Costumers ought to also pay attention to online shopping, because the label information is not always available or updated (47). Finally, food allergic patients shouldn't buy a product if they have doubts about their composition and safety (34).

Eating out

Schools

Food allergy is a common issue in school setting (59), and the risk of reactions at schools is a major concern for parents of children with food allergy (5). Parents should ensure that children bring safe snacks from home and understand the risk of trading or accept food (27).
Regarding the school role, there are some additional measures, like providing food allergy education as part of science curriculum (60). It is also important to teach that it is wrong to tease or bully people with food allergies (61). Additionally, school can inform children about programs like *Be a PAL: Protect A Life*™ which can help them learn how to be a good friend to people with food allergies (62).
Other resolutions can be to provide, when possible, individually wrapped food, clearly labeled food products or to designate an allergy-friendly seating during meals (open to any child with safe food) (63,64). Daily menus with allergen information shall be provided to the families (28,64). Hence, food allergens shouldn't be used in craft projects if there is a food allergic child in the class (63) and non-food incentives shall be used as prizes or gifts (64).
Younger children should be more supervised, especially in terms of cleaning practices (63) and responsible persons ought to be designated to manage the rapid and proper access to epinephrine auto-injectors (one of these should belong to the cafeteria staff) (64). Appropriate food handling procedures in canteens is also crucial to avoid accidental exposures and, given that, it is important that cafeteria / food service staff receive proper training (28,63).
Additionally, it is pivotal to provide adequate information to school personnel like teachers, substitute teachers and field trip personnel (5,28,29,63). A directed handbook about food allergies edited by a government authority, like the one made in Portugal (65), could be an important tool.

Family and Friends' houses

Eating out also includes eating at family or friends houses and this is a frequently disrupted activity for food allergic individuals (29). Eating in a friend's house is particular important to adolescents, as they realize that difficulty of socializing with friends is one of the main effects of having an allergy (29,32). Given that, family and friends of food allergic individuals should receive information about the disease and allergen avoidance tools and be properly trained to deal with emergencies (29,66).

Restaurants

Restaurants also point challenges for food allergic costumers (18,28,50,67) and some individuals count it as a principal restriction in their daily routine (54).
In a study of Worth et al, 37% of the food allergic respondents said that the question that concerned them most was the limitation of not being able to go to restaurants (32). To minimize

the potential risks, food allergic individuals can take some measures as always carrying epinephrine auto-injector (if it was previously indicated by a physician), communicating with chef / restaurant manager about food allergy and their needs regarding the ingredients and cooking methods (11, 28), and being aware of restaurants that present a particular danger like Asian food restaurants (68), ice cream shops, bakeries and seafood restaurants (69), considering the food allergens. Buffets could also be a problem for food allergic individuals essentially due to the risk of cross-contact (44, 50, 69).

Communication

Having meal away from home requires a proper searching about the conditions of a restaurant, and consequently a direct communication between the consumer and the restaurant personnel (11,28,70). Wanich et al. identified, in a study, communication problems for both client and restaurant personnel (35). So, in every visit to a restaurant, patients and their families must know how communicate about food allergy and their needs regarding the ingredients and cooking methods (11,28).

Carrying a *chef card*, that outlines the foods that require avoidance and other information, is another important and common strategy (47). Additionally, they should chose a day and time when restaurant staff is not busy, so they could be more alert and attentive (69).

Food allergic costumers shall prefer simple dishes (for example baked potato instead of purée), avoid sauces or garnishes and be careful with desserts (44,68,69). On the other hand, food allergic costumers need all the restaurant staff to be proper informed and trained about food allergy (44, 68). Regarding this, studies have stated a particularly worrying discrepancy between the personnel's knowledge about food allergy and their comfort level in providing a safe meal (67,71,72).

Restaurants should have their menu as complete as possible, regarding the food allergy questions (73). For instance, an *apple cake* should be described as *apple cake (with nuts)* or *cottage pie* should be described as *cottage pie (chicken, puree, egg)* (74).

Additionally, it is very important to develop updated standardized recipes that include identification of food allergens and also potential of cross-contact based on restaurant and cooking procedures and HACCP implemented (75).

According with the EU legislation - Regulation (UE) n. 1169/2011, that came into force in December 2014, the clear identification of the 14 "major allergens" is mandatory, according to Annex II. This Regulation establishes the general principles, requirements and responsibilities governing food information, and in particular food labelling, including labelling of certain substances causing allergies. It states that the provision of food information shall pursue a high level of protection of consumers' health and interests, by providing a basis for final consumers to make informed choices and to make safe use of food. Given that, it also lays down the means and procedures to guarantee the right of consumers to information, and shall apply to food business operators at all stages of the food chain, including foods delivered by mass caterers, foods intended for supply to mass caterers, and food served by transport undertakings when the departure takes place on the territories of the Member States to which the Treaties apply (41).

Besides the obligation to provide allergen / information in a visible and legible way (41), if they are asked for, restaurant staff should give clear information on potential allergens and complete disclosure of the dishes' ingredients (even if there was a *secret* one) (44,73). Another fundamental rule in food allergy is never guessing. If the employee does not have the total assurance of the ingredients, he must notify the customers and help them to choose another dish (44,73). Furthermore, it is important that restaurant personnel ensure the total cleanliness of the table and chairs (including highchairs) as for skin contact with residual food can provoke a reaction (44,73). Moreover, the table chosen for these costumers should be as far as possible from the kitchen in terms of avoiding inhaled contact with cooking vapors and out of the operational way (44).

Service

Regarding service in restaurants, cross-contact is a main problem (28,44), especially because these establishments have constrains that increase the risk: large variety of allergenic foods in the same and generally constrained facility, constant sharing of surfaces and utensils, and simultaneous preparation of many dishes (51).

Cross-contact happens when a food that isn't an allergen or does not contain itself any allergen, comes into contact with an allergenic food. As a result, their proteins will mix and a food that was *safe* for an allergic individual then becomes risky (34,69). Cross contact can occur directly (when one food is placed above another) or indirectly (through hands or cooking utensils) (34,44). A practical scenario could be serving to a shellfish allergic individual a chicken that was grilled and handled with the same utensils that were used to cook a shrimp (34).

Cross-contact is a serious concern for people with food allergies, given that it is one of the main sources of undeclared or hidden allergens (51,74). Anibarro et al. have reported that hidden allergens accounted for 21% of all food allergic reactions (49). Additionally, in a restaurant, cross-contact is more likely to lead to high-dose exposures than at home, which may cause more severe reactions (51).

Regarding this issue, staff must be instructed about food preparation and service techniques to avoid cross contact (49). For instance, they should not use the same utensils to prepare, cook, plate and distribute of different meals. Water in which foods

are cooked and oils used in fried foods shouldn't be shared, too (44,69). Staff should also pay attention to the cleanness of all the utensils (including those used to wash or clean), tableware, storage containers and fridge / freezer, kitchen bench and kitchen appliances (grill, microwave, toaster, hand blender and chopper) (11,44,69,73). The hygiene of the employees and their uniforms is another key point and kitchen staff shall use non-latex and clean gloves for working (44).

Whenever possible, meals for food allergic costumers should be prepared first, and it is important to note that all the ingredients, even those used in small amounts (like flour to thicken a sauce or ingredients included in marinades) can provoke a reaction (44). Further, and given that there are some dishes that food allergic patients will certainly avoid (fried and grilled food due to the risk of cross-contact) (69), it is important that the restaurant considers the possibility of other menu options (44). Finally, if an error occurs, the solution is to discard the dish instead of only removing the portion that is believed to be contaminated (44,69,71).

During the service, the plate of a food allergic client should be delivered separately in order to avoid cross-contact and immediately after the preparation (11, 44, 69).

Travelling and vacation

Food allergies affect food allergy individuals' vacations. Food allergic individuals' families commonly restrict the number of vacations they take, and some of these have never vacationed (36,76). The chosen destination is likewise affected, as patients say that they avoid for example Asian countries due the high risk of local cuisine (76). Additionally, food allergic adolescents cited difficulty travelling / going on overnight trips as one of the main effects of having an allergic reaction (32). It is also usual that these individuals avoid mainly ships and planes (36). Concerning this, and given that airplane is frequently needed in everyday context, the issue of safe air travel is particularly anxiety provoking (37,68). Studies have reported that about 10% of the food-allergic passengers have already experienced a reaction onboard (37,77). It was also mentioned that one of the occurring reactions led to emergency landing (77).

Additionally, Comstock described that among 471 patients with peanut, tree nut or seed allergy, 9% reported reactions during a flight, 10% of which had more than one reaction (78).

Regarding in-flight reactions, and given that there are fewer resources in an airplane which could lead to an undertreated reaction (37,79), cabin staff should receive appropriate training about this issue, and airlines ought to implement some measures concerning food allergic passengers' safety and well-being (28,79).

Many North-American airlines have implemented some resolutions as the elimination of the distribution of peanuts during the flight, concerning the high prevalence of this food allergy. However, and taking into account that nothing prevents other passengers to bring their own snacks on board, the risk of exposure is always present (37,80).

This risk also still present on the "buffer zone" or "peanut free area", another measure that provides a zone reserved for food allergic passengers around which peanut (or other allergen) cannot be consumed (79). In this case, the ventilation system could ensure the dispersion of peanut particles and individuals may have a reaction by inhalation (37,79,80).

For passengers, the approach to eating on an airplane should be the same as that for any restaurant. They should contact the airline before the trip and inform the cabin staff on the day (28,81). It is also important for food allergy passenger to carry their adrenaline kits, in their original packaging, in the aircraft cabin and not into the luggage hold (28,37,80,81). However, since 11th September 2001, an allergic patient can theoretically be denied to use injectable epinephrine in the plane, so all the situations should be clarified with the airline (80,81). Further, it is recommended that patients have a letter written by their physician, containing all the information about their medical conditions and needs (70,80,81). Besides that, food allergy individuals shall ask airline to bring their own food onboard in order to avoid potentially unsafe airline foods (11,68). Another important advice is to inspect the cleanness and the presence of residual foods in the seats, especially in the case of food allergic babies or toddlers (11,81). Additionally, the avoidance of airline pillows or blankets and the request that other near passengers don't consume products with the implicated allergen, could be important tools (37).

At the destination, food allergic individuals should choose accommodations where self-cooking is possible (11,70,76). Chain restaurants could be another option, given that they are likely to use the same ingredients and to follow the same recipe, and that a growing number is allergy-aware (69). It might also be important to take an allergy information card in the host language (28,68,70) and to verify the food allergen labelling laws of their destination country before buying packaged foods (48).

The availability of medical care should be taken into account when food allergic individuals decide their destination and what to eat (68,76).

Critical Analysis and Conclusions

The everyday life of a food allergic individual presents several challenges. These patients are constantly measuring risks associated with going shopping, buying foods, eating at school or in a restaurant, or travelling, in order to avoid an accidental exposure. For effective and personalized food allergen avoidance, essential information is required, as well as adequate training of the patients to understand the labels and to communicate with food

suppliers (20). The implementation of the tools of avoidance suggested by this review is essential to manage food allergy outside home and avoid serious allergic reactions. On the other hand, training for restaurants, schools, food industry and aircraft staff is also desirable, as well as a greater awareness of the disease.

The increasing recognition of the importance of the relationships between well-being and health has changed the way health and diseases are treated (25). Thus, today the intention is to assist and advise the patients in their disease's management, for what concerns a lesser impact on their daily life. Accordingly, one of the NIAID-sponsored expert panel's guidelines is that "*patients with food allergy should be provided with information on food allergen avoidance and emergency management that is age and culturally appropriate*" (1).

However, it is important to note that despite that some of the advices given in this paper are apparently easy to implement, there is a gap in studies regarding the efficacy of this measures.

A study of Ewan and Clark is one of the few works that exists regarding the efficacy of an intervention to help patients in peanut allergy management and avoidance. Considering that the management plans have reduced the frequency and severity of reactions, counselling and information about allergen avoidance (understanding labelling, eating in restaurants or travelling abroad) are crucial for food allergic individuals (82). Another study defends the important role of the nutritionist and nutritional therapy in group in this educational process (83).

Additionally, and taking into account that there is also paucity in literature about accidental exposure, studies are needed to get more information about frequency and severity of unexpected allergic reactions to food. This data will be important to direct and optimize strategies to support patients in managing their food allergy; to prevent accidental exposure as much as possible and to increase awareness and knowledge in restaurants, airlines and food industry (13). Further, these results would be very important to provide more confidence and compliance for food allergic individuals to deal with their disease.

Other strategies that could be adopted to ensure the well-being of patients include the development of adapted recipes (34), as well as the creation of specialized restaurants. This strategy assumes particular importance if we take into account that some food allergic adolescents say that they need willpower to resist food they knew they should not be eating. Additionally others say that they often look for enjoyable and safe alternatives so they can feel better (84).

The creation of food products that suits food allergic individual's demands, especially those aimed to be consumed by children could be relevant, too. Moreover, now it is well recognized that the protection of food allergic individuals from accidental exposure and reactions is a shared responsibility (20).

With this review, it is also highlighted that proper and directed tools and more training for restaurant and aircraft staff are desirable, and that it would be important an investment in the development and implementation of effective allergen management strategies for food industry (20,26). The implementation of effective tools is essential to manage food allergy outside home, to avoid serious allergic reactions and to minimize the disease's impact on individuals' quality of life. Additionally, if the right measures are taken, and if all partners work together to ensure and improve the support services for food allergic individuals, these patients can have a normal, healthy and joyous life.

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Probiotics and refractory chronic spontaneous urticaria

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Summary

Background. *In chronic spontaneous urticaria (CSU) first-line therapy with an antihistamine-based regimen may not achieve satisfactory control in patients. Thus, a continuing need exists for effective and safe treatments for refractory CSU.* ***Aim.*** *To evaluate the clinical efficacy and safety of an intake of a combination of 2 probiotics (*Lactobacillus salivarius *LS01 and* Bifidobacterium breve *BR03) in patients with CSU who remain symptomatic despite concomitant H1-antihistamine therapy.* ***Methods.*** *This report analyzes the effects of therapy with two probiotic strains on the clinical progress of 52 unselected patients with difficulty to treat CSU underwent to medical examination in two Italian specialist urticaria Clinics between September 2013 and September 2014. A mixture of* Lactobacillus *LS01 and* Bifidobacterium *BR03 were administered in each patient twice daily for 8 weeks. To evaluate patients' improvement with probiotics, urticaria activity score over 7 days (UAS7) was used at baseline and at week 8 in addition to a 5-question urticaria quality of life questionnaire.* ***Results.*** *Fifty-two patients with CSU were included in this study (10 male and 42 female, age range 19-72 years). Mean disease duration was 1.5 years. Fourteen patients discontinued treatment, so evaluable population consisted of 38 patients. Nine of the 38 patients experienced mild clinical improvement during probiotic treatment (23.7%); one patient reported significant clinical improvement (2.6%) and one patient had complete remission of urticaria (2.6%). Twenty-seven patients did not have improvement in symptoms (71.1%). No side effects during the course of therapy were reported.* ***Conclusions.*** *A combination of* Lactobacillus salivarius *LS01 and* Bifidobacterium breve *BR03 administered twice daily for 8 weeks might reduce the symptoms scores and improve quality of life scores in a part of patients with CSU who remained symptomatic despite treatment with H1 antihistamine mostly in subjects with allergic rhinitis.*

Introduction

Chronic spontaneous urticaria (CSU) is classically defined as the occurrence of spontaneous wheals on most days for more than six weeks. It is a common skin condition that affects 0.1-3% of people in the USA and Europe (1).

The course and duration of CSU are highly variable and unpredictable. Spontaneous remission may often occur within 12 months, but a substantial number of patients may have symptoms lasting periodically for years, or suffer irritating symptoms such as pruritus for decades (2). CSU is frequently a disabling disease due to the persistency of clinical symptoms, the unpredictable course and negative influence on the quality of life, as it can cause sleep disruption, fatigue, social isolation, energy loss and emotional / sexual disturbances (3). The goal of treatment

in CSU is to ensure rapid and prolonged control of the symptoms and a rapid return to normal social activities.
Symptomatic treatment for CSU is the most frequently used form of management, and a step-wise approach is recommended (4). Modern second-generation H1-receptor antagonists are the primary treatment at licensed doses (5,6), and updosing is second-line treatment. First-line therapy with an antihistamine-based regimen may not achieve satisfactory control in 5% to as many as 50% of patients with CSU (7). Those with refractory CSU require treatment with H1-antihistamines increasing doses by up to 4 times; if symptoms persist, a trial of omalizumab, cyclosporine or montelukast (4,8) as add on therapy is recommended; frequent exacerbations may be treated with systemic steroid. However, the toxicities and adverse events associated with cyclosporine and long-term steroid exposure should be considered carefully (9). Thus, a continuing need exists for effective and safe treatments for refractory CSU; trials of several novel therapeutics are in progress.
It was seen that *Lactobacillus salivarius* LS01 and the combination of 2 probiotics (*Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03) have the capability to reduce the release of pro-Th-2 cytokines from THP-1 cells, favouring an improvement in T-helper cell (Th)1/Th2 (10,11).
Th2 cells play a critical role in the pathogenesis of allergic reactions and in the production of IgE antibodies.
In CSU, IgE antibodies, FcεRI, and mast cells are likely to play essential pathologic roles, although the causative factors have not been identified (12). The aim of this study was to evaluate the clinical efficacy and safety of an intake of a combination of 2 probiotics (*Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03) in patients with CSU who remain symptomatic despite concomitant H1-antihistamine therapy.

Materials and Methods

This report is an analysis of the effects of therapy with two probiotic strains on the clinical progress of 52 unselected patients with difficult to treat CSU, who previously underwent medical examination in two Italian specialist urticaria Clinics (Unit of Study on Urticaria / Angioedema, Policlinico, Bari, and Allergology Unit, Miulli Hospital, Acquaviva delle Fonti) between September 2013 and September 2014. To be started on probiotic therapy, patients had to have shown to be unresponsive after almost one month of H1-anthistamines treatment.
In all enrolled patients, a diagnosis of CSU was made by a careful history and detailed physical examination, submitting them to clinical, laboratory and instrumental investigations according to individual clinical history and findings in each patient. Tests included: urinalysis, routine laboratory evaluation (including complement C3, C4 and C1 inhibitor antigenic level, thyroid function test, antithyroid autoantibodies, antinuclear antibodies, rheumatoid factor, serum immunoglobulins, circulating immune complexes, cryoglobulins, stool screening for blood, parasites and yeast, serology for viral, bacterial and parasite antibodies, serum electrophoresis, gastroscopy, biopsy and enzyme-linked immunosorbent assay for specific anti-Helicobacter pylori IgG antibodies), X-ray studies (including dental series, sinus series and chest X-ray) and sonography of the upper abdomen. Skin prick tests were performed with common available foods and inhalants (Stallergenes, Milan, Italy). In some cases, a "prick-prick test" with fresh raw food was made. Measurement of total IgE level was made (UniCAP, Thermofisher, Milan, Italy) and specific serum IgE according to patients' anamnesis (UniCAP, Thermofisher, Milan, Italy).
Drug-related etiology was established on the basis of the criteria laid down by the protocols in literature (13). Briefly, the methods used to evaluate patients with suspected drug-induced urticaria were a detailed history, withdrawal of the suspected drug, and in some cases in vivo and in vitro testing. In order to evaluate the role of foods and additives, single blind placebo-controlled in vivo provocation tests with foods and additives were performed when necessary. Autologous serum skin tests, were performed as previously reported (14). So, in these patients inducible urticarial alone, urticaria caused by medications, insect bites, food or other known causes were excluded. In addition, patients with significant concomitant illness (e.g. malignancies or psychiatric, hepatic, endocrine or other major systemic diseases) were also excluded.
After the CSU diagnosis, all patients had received second generation H1-antihistamines at up to twofold higher than the licensed dose in an attempt to control their condition. Some patients had even received three or more different antihistamines. Eighteen and nine patients had previously required corticosteroids and montelukast, respectively, to control symptoms.
Patients were administered twice daily for 8 weeks a marketed oral probiotic (Bifiderm®, Bayer S.p.A, which is a mixture of *Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03 at a dose ≥ 1x109 colony-forming units (CFU)/g each in maltodextrin).
Throughout the treatment period, participants were required to maintain stable doses of the previous therapy with H1-antihistamines.
Each patient was examined by the physician 3 times over the 8-week period: this included (apart from the initial screening visits), a 1st visit at the start of treatment with the probiotic state; a 2nd visit after 4 weeks of treatment; and a final visit after 8 weeks (end of treatment).
Throughout the study and one week before starting the probiotic state, all patients recorded their symptoms in a daily diary (pruritus and number of wheals). At each clinical visit the patient's diary was reviewed, the patient was interviewed as to the

event/s occurring in the previous week/s, and a physical examination was performed.

Baseline severity was determined by urticaria activity score over 7 days (UAS7) 1 week before probiotic treatment. UAS7 is a simple patient-reported scoring system that captures the severity of pruritus and number of hives during 1 week (15). Intensity of pruritus (range, 0 [none] to 3 [severe]) and the number of hives ratings (range, 0 [none] to 3 [> 12 hives]) were recorded daily (maximum, 6 points per day). Scores were then summed for 1 week to represent the UAS7 (scale, 0-42). All patients had a UAS7 of 6 or greater despite antihistamine therapy. The primary end point was the change from baseline to week 8 in the UAS7. The responses to the probiotic state were described as follows: "complete response" was defined as a reduction of 90% or more in the UAS7, "significant improvement" as a reduction in the UAS7 of 90%-30%, "mild improvement" as a reduction in the UAS7 of 30%-10% and "no significant improvement" as less than 10% reduction in the UAS7.

A 5-question urticaria quality of life questionnaire was administered at each clinical visit, evaluating the following domains: cutaneous symptoms, emotions, practical problems. The questions were: "Over the last week, how itchy, sore, painful or stinging has your skin been? Over the last week, how embarrassed or self-conscious have you been because of your skin? Over the last week, how much has your skin influenced the clothes you wear? Over the last week, how much has your skin affected any social or leisure activities? Over the last week, has your skin prevented you from working or studying? If "No", over the last week how much has your skin been a problem at work or studying?". These are part of the Dermatology QualityLife Index (16).

Patients scored their response to each question on a 4-point scale ranging from 0 (no problems) to 3 (severe problems).

Safety and tolerability were assessed on the basis of the adverse events referred or changes in vital signs, and physical examination findings.

Approval from the Ethics Committee of the hospital was not necessary, because the analyses were performed on data recorded during the routine treatment of patients. Patients provided oral informed consent to have their data included for analysis.

Results

Fifty-two patients with CSU were included in this study (10 male and 42 female, age range 19-72 years). Mean disease duration was 1.5 years (range 0.3-9.4 years). Twenty-four of these subjects had a history of angioedema. Twelve of the 52 patients had to have a documented history of seasonal or perennial allergic rhinitis related to positive skin prick test and/or laboratory tests.

Fourteen patients discontinued treatment. The reasons for discontinuation were: non-compliance (n = 3); and lack of desire to continue because of no improvement in symptoms (n = 11).

The evaluable population thus consisted of 38 patients. A total of 18 patients (44%) were classified as having a suspected chronic autoreactive urticaria demonstrated by a positive autologous serum skin test.

Nine of the 38 patients experienced mild clinical improvement during probiotic treatment (23.7%). One of the 38 patients experienced significant clinical improvement (2.6%). One patient had complete remission of urticaria (2.6%). This female patient had a UAS7 of 8 despite antihistamine therapy, and the duration of urticaria was 6 months. Then, a total of 11 subjects (28.9%) showed improvement on Probiotic therapy (**table 1**).

In this group, at week 8, mean 5-question urticaria quality of life questionnaire score decreased from baseline (1 week before probiotic treatment) by 2.46 points. Twenty-seven patients did not have improvement in symptoms (71.1%) and eleven of them required short courses of prednisone for symptom relief.

We compared the characteristics of 11 patients with improvement in symptoms with those of 27 patients without improvement in symptoms.

In subjects with improvement in symptoms emerged only a high prevalence of allergic rhinitis (8 of 11) than in the group of patients without improvement of symptoms (2 of 27).

No patient reported any side effects during the course of therapy in all study groups.

Discussion

CSU, one of the most frequent skin allergy diseases, is a heterogeneous condition, and prognostic factors for each treatment are not well known. CSU is a disease that is particularly difficult to treat. Although non-sedating antihistamines are recommended as first-line agents, a substantial proportion of patients remain poorly responsive to these agents even if H2-receptor antagonists and/or leukotriene pathway inhibitors are added (17). Such patients are often treated with corticosteroids or cyclosporine or omalizumab (4), and alternatives to these agents would be a welcome addition if efficacy could be shown with an acceptable tolerability profile. Thus, a continuing need exists for effective and safe treatments for refractory CSU.

In this study we evaluated the clinical efficacy and safety of an intake of a combination of 2 probiotics (*Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03) in patients with CSU who were refractory to conventional treatment.

In a group of 11 subjects, after Probiotics intake, a reduction of disease activity and improvement of patients' health-related quality of life were observed. From a questionnaire administered to urticarial patients, O'Donnell et al. (3) established that the disability described by patients is comparable to that of patients with ischemic heart disease. Successively, Finlay et al. (16) developed the Dermatology Life Quality Index (DLQI). They used it to measure and compare the disability induced by a variety of

common dermatological conditions and suggest that the questionnaire can be administered before and after treatment interventions, to serve as an indicator of treatment efficacy.

Because of the relatively small patient population in our study, it was difficult to determine any patient characteristics that were predictors of response to Probiotic supplement. The presence of thyroid autoantibodies, angioedema, positive ASST, and age did not appear to predict response. The presence of allergic rhinitis in CSU patients seemed to be a possible predictor of response to Probiotics.

The role of probiotics in regulating intestinal health has been widely studied for over a century. Modulation of the intestinal microbiota is one of the important functions of probiotics, which is deeply associated with the modulation of the intestinal immune system, improving bowel movement and decreasing allergy (18,19). However, in recent years, several lines of evidence suggest that some bacterial probiotics can modulate the skin immune system (20). In human clinical trials, probiotic supplementation showed potential in the relief of atopic dermatitis and dry skin (21).

There is evidence suggesting that alteration of the composition and/or size of the gut microflora may modulate the IgE response to allergens (22). Because modern lifestyles have contributed to changes in the composition of the intestinal microflora, diet supplementation with probiotics may counterbalance the Th-2 activity by promoting Th-1 cytokines production and downregulate IgE production via inhibition of IL-4 and IL-5 production (23). Additionally, it was showed that *L. paracasei* NC 2461 induced development of a population of CD4+ T cells that produced TGF-β and IL-10 (24), which could downregulate IgE production (25).

It was seen that *Lactobacillus salivarius* LS01 and the combination of 2 probiotics (*Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03) reduced the release of type 2 cytokines [interleukin (IL)-4 and IL-13] and induced an improvement in the T-helper cell (Th)1/Th2 ratio. This probiotic formulation upregulates Th1 functions and down regulates Th2 and Th17 activity, improving Th1/Th2 and Th17/Treg ratios (10,11).

Recent evidence suggests that helper T cells (Th2) play a triggering role in the activation / recruitment of IgE antibody producing B cells, mast cells and eosinophils (26).

In cases of CSU, in which autoreactive IgG antibodies against FcεRI, IgE, or both or autoreactive IgE antibodies against autoallergens are found, these autoantibodies are causative factors, and IgE, FcεRI, and mast cells are unambiguously at the centre of the pathologic process. For the remaining cases of CSU, IgE, FcεRI, and mast cells are also likely to play essential pathologic roles, although the causative factors have not been identified.

Autoimmune processes might be the primary cause of most cases of CSU. Thus, for those cases with a clear autoimmune

Table 1 *- Characteristics of 11 patients who showed improvement in symptoms.*

No	Sex	Age, y	Duration of urticaria	Angioedema	Anti thyroid Antibody	Previous Treatment	ASST	Allergies (Rhinitis)	Effect
1	F	41	2 y	Yes	+	H1 Pred Mont	+	Parietaria	↑
2	F	36	1 y	No	-	H1	-	Grass, Olive	↑
3	F	27	5 mo	No	-	H1	-	Cypress	↑
4	F	35	3 y	Yes	+	H1 Pred	+	Grass, Olive	↑
5	F	39	7 mo	No	-	H1	-	No	↑
6	M	57	1 y	No	-	H1	-	No	↑
7	F	21	2 y	Yes	+	H1 Mont	-	Grass, House dust mite	↑
8	F	46	4 mo	No	n.d.	H1	-	No	↑
9	M	44	6 y	Yes	n.d.	H1 Pred Mont	+	Grass, Olive, Cypress	↑
10	F	39	1 y	No	-	H1 Pred	+	House dust mite, Grass	↑↑
11	F	47	6 mo	Yes	+	H1	+	House dust mite	↑↑↑

F = female; M = male; y = years; mo = months; H1 = H1-antihistamines; Pred = prednisone; Mont = montelukast; ASST = autologous serum skin test; Effect = over change in clinical symptoms after Probiotics treatment; mild clinical improvement (↑), significant clinical improvement (↑↑), complete remission of urticarial (↑↑↑); n.d. = not done

cause, the reduction of the IgE by the action of Probiotics yields the observed therapeutic efficacy. Even for those cases that involve autoimmune response and autoreactive IgE antibodies subtly, they still involve the central pathologic axis of IgE-FcεRI-mast cells, and Probiotics similarly render therapeutic effects (27,28).

The inflammatory response in the nasal mucosa in subjects with allergic rhinitis challenged intranasally with an allergen includes an immediate IgE-mediated mast cell response as well as a late-phase response characterized by recruitment of eosinophils, basophils, and T cells expressing Th2 cytokines including IL-4, a switch factor for IgE synthesis, and IL-5, an eosinophil growth factor. Recent advances have suggested that additional pathways may contribute to the pathophysiology of allergic rhinitis including local synthesis of IgE in the nasal mucosa (29).

Several studies demonstrated that Probiotics may alleviate the symptoms caused by allergic rhinitis. A meta-analysis described the results of 12 randomized clinical trials with probiotics in AR: in nine of the 12 trials, there were some improvements in clinical outcomes including nasal or ocular symptoms (30).

These data suggest that Probiotic therapy might be more effective in those patients with IgE-mediated allergic rhinitis associated with CSU.

The safety profile for the probiotic strain over 8 weeks of treatment in our patients was consistent with previous observations in patients treated with probiotic supplement (31).

Future studies should include a larger number of patients and have a double blind placebo-controlled design. The allergic setting is linked to the disruption of the Th1/Th2 balance with a Th2 profile; as a consequence, the production of IL-4, Il-5, or IL-13 by Th2 lymphocytes contributes to the development and maintenance of the allergic response. Probiotic strains, like *Bifidobacterium breve* BR03 and *Lactobacillus salivarius* LS01, able to induce massive secretion of IL-10 by human PBMCs of subjects with allergic asthma and to down-regulate the secretion of TGF-β, IL-13, and IL-17 in asthmatic subjects, may lead to the rebalancing of Th1/Th2 ratio and to the improvement of allergic symptoms and could limit the proinflammatory response, simultaneously improving the process of maintaining the state of tolerance to external antigens (32).

In addition, further studies are needed to help better identify clinical and laboratory predictors of response.

In conclusion, our study suggests that a combination of 2 probiotics (*Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03) administered twice daily for 8 weeks might reduce the symptom scores and quality of life scores in a part of patients with CSU who remained symptomatic despite treatment with H1 antihistamine, mostly in subjects with allergic rhinitis. The probiotic approach might represent a new well tolerated option in the treatment of CSU.

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Satisfaction and perceived effectiveness in patients on subcutaneous immunotherapy with a high-dose hypoallergenic pollen extract

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Key words
Allergoid; subcutaneous immunotherapy (SCIT); hypoallergenic; high-dose; effectiveness; perceived effectiveness

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Summary
This study was designed to determine the level of satisfaction, tolerance and perceived effectiveness by patients in the first pollen season after starting treatment with Alergovit®. For this purpose, a nationwide, retrospective, multicentre and cross-sectional observational study was carried on 256 patients. Perceived effectiveness by the patients was measured using a visual analogue scale and was clinically significant in 92.4% of the patients. The satisfaction level was evaluated with a specific questionnaire. 32.5% of the patients were totally satisfied with Allergovit® and 48.8% reported a high degree of satisfaction. The treatment was well tolerated by 99.2% of the patients. Our results demonstrate that subcutaneous immunotherapy with Allergovit® is effective and well-tolerated in routine clinical practice.

Introduction

Allergen immunotherapy (AIT) is the only treatment that may affect the natural course of allergic disease and prevent the development of asthma in patients with allergic rhinitis (1). Its efficacy in treating IgE-mediated allergic rhinoconjunctivitis and bronchial asthma has been clearly demonstrated (2,3).
To achieve the greatest possible efficiency, it is recommended to perform AIT for at least 3 years and to start it in an early stage of disease. Moreover, the treatment schedule should be convenient for patients in order to get a good treatment adherence (4).
Matricardi *et al.* (5) recently published a comparative review of various meta-analyses, which included at least 5 double-blind, randomised, placebo-controlled studies, with the aim of assessing the short-term efficacy of both symptomatic medication and subcutaneous immunotherapy (SCIT) for the treatment of seasonal allergic rhinitis. The authors concluded that, based on nasal or total symptom scores, SCIT is at least as effective as symptomatic medication in patients with seasonal allergic rhinitis in the first pollen season after the start of therapy (5).

Short-term efficacy of subcutaneous high-dose hypoallergenic pollen extracts (Allergovit®) as well as its safety using up-dosing cluster schedules, has been widely demonstrated by several previous studies (6-8).

Nevertheless, there is a clear need to supplement the results from clinical trials with "real-life" studies to provide us with specific effectiveness and safety data under routine clinical practice conditions, in addition to information on other subjective aspects such as degree of satisfaction with the treatment, since this will affect adherence to (4,9) and, consequently, the effectiveness of the therapy (10,11).

A German prospective, observational study was recently published which confirms effectiveness and safety of SCIT with Allergovit® under routine clinical practice conditions but provides no data on subjective aspects perceived by the patients (12).

The objective of this study was to determine the level of satisfaction, tolerance and effectiveness as perceived by patients on treatment with Allergovit® after the first pollen season, within the routine clinical practice criteria of the participating investigators.

Materials and Methods

Allergen extract composition

The tested product, Allergovit® (Allergopharma KG, Reinbek, Germany), is a standardised high-dose hypoallergenic aluminium hydroxide-adsorbed depot preparation modified with formaldehyde. This pollen allergen preparation is available in two different concentrations: strength A (1,000 TU/ml), and strength B (10,000 TU/ml). The manufacturer's recommended maintenance dose is 0.6 ml of B strength (6,000 TU). The major allergen contents in strength B are 41.66 μg_{eq}/ml group 5 allergen in *Graminaceae* formulations and 18.33 μg_{eq}/ml Ole e 1 in 100% *Olea europaea* formulations.

Study design

This was a nationwide, retrospective, multicentre, cross-sectional observational study with the participation of 29 investigators distributed across 7 Autonomous Regions in Spain. It was approved by the ethics committees of the participating hospitals and notified to the Spanish Agency of Medicines and Medical Devices (AEMPS). It had several objectives, the primary one being to determine the level of satisfaction, adherence, tolerability and perceived efficacy of patients on Allergovit® treatment. The secondary objective was to examine demographic and clinical variables that could be related to patient satisfaction with Allergovit® treatment.

Patients should have a previous diagnosis of pollen-induced IgE-mediated rhinitis and/or bronchial asthma before being included in the study. The diagnosis of allergic rhinitis was based upon the concordance between the typical symptoms of allergic rhinitis (rhinorrhea, sneezing, nasal obstruction and pruritus) and diagnostic tests (demonstration of allergen-specific IgE in the skin (immediate-hypersensitivity skin tests) or the blood (specific IgE).

A diagnosis of asthma was made following a clinical assessment of symptoms (dyspnea, cough, intermittent and variable wheeze, chest tightness, and shortness of breath) and demonstration of variable airflow obstruction, which was assessed by performing pre-bronchodilator and post-bronchodilator spirometry. An improvement of forced expiratory volume in 1 second (FEV1) and/or forced vital capacity (FVC) of greater than 12% or 200 mL was considered a significant BD response, consistent with asthma.

Moreover, since the focus of this study was to determine summary statistics characterizing the study population, no calculation to determine sample size was performed. It was considered appropriate to include 250 patients who were representative of the Spanish population. To do so, each investigator who participated in the study had to consecutively include roughly 10 patients satisfying the inclusion / exclusion described above; it was estimated that it would be appropriate to involve a minimum of 25 investigators.

During the observational period, the investigators gathered data from those patients who met the inclusion criteria for the study (age 5-65 years, pollen-induced IgE-mediated rhinitis and/or bronchial asthma) and who, as part of routine practice, had been treated with Allergovit®, and had a follow-up visit in the first 6 months of treatment, for those on a perennial administration protocol; or who had completed at least one therapeutic cycle, if the administration was preseasonal.

For the evaluation of perceived effectiveness, patients assessed their conditions on a visual analogue scale from 1 (worst condition) to 100 (best condition). A clinically relevant improvement was defined as improvement by at least 20 points between the self-assessments made before they started the treatment and at the time of the evaluation (7).

In addition, effectiveness was also measured as a continuous variable resulting from the difference between state of health at the time of the study and before starting the treatment, based on the data collected on the patient chart review.

Patients' satisfaction with SCIT with Allergovit® was evaluated based on a specific questionnaire (included in the appendix section). A paper Case Report Form (CRF) including several sociodemographic variables (patient's date of birth, educational level, occupational status, nationality and marital status) and clinical variables (previous treatments, family history, severity and Allergovit® treatment) was prepared, also including treatment adherence.

The variables included in the questionnaire were: the need for treatment to be administered subcutaneously and for this to be done by a healthcare professional, the impact having to go to the health centre had on subjects' daily routine, the level of improvement in symptoms with treatment and overall satisfaction with treatment. A Likert scale was used to objectively evaluate the questionnaire's variables, with 0 representing strongly unsatisfied and 5 totally satisfied.

Data analysis and statistical techniques

A descriptive analysis was performed on the whole sample, calculating the mean and standard deviation (SD) as descriptive statistics for the quantitative variables with normal distribution, and median and interquartile range if the distribution was not normal. Proportion was used for categorical variables.
The data collected in the CRFs were entered into a database using simple data entry for statistical analysis. The database was validated to ensure its quality prior to the start of the analysis, first by using a frequency analysis to detect extreme or impossible values and then by analyzing any intra-CRF inconsistencies. Bivariate association techniques were used to study the relationship between the main dependent variables (satisfaction, tolerability and effectiveness) and the explanatory variables such as patient age, diagnosis and the allergy specialist's workplace. The Pearson correlation coefficients were calculated for dependent variables with sufficient sample size (designing multivariate models to determine the associated factors).
The level of significance considered in the statistics calculated was $p < 0.05$.

Results

Between September and November of 2012, data were collected from patients diagnosed with pollen-induced IgE-mediated rhinitis and/or bronchial asthma who had started treatment with Allergovit® and met the inclusion and exclusion criteria stated in the protocol.
A total of 256 patients were included. In terms of diagnosis, 246 of the patients (96.5%) had rhinitis and 163 patients (63.9%) had bronchial asthma. 81.6% of the SCIT preparations contained grasses, 36.6% olive and 7% other allergens (trees and weeds) (table 1).

Perceived effectiveness by the patients

Patients assessed their condition on the visual analogue scale as improved by mean 33.5 points (40 to 73.5 points; $p < 0.001$), improving by between 30 and 50 points in 51.6% of the total population (132 patients) and being clinically significant (improving by over 20 points) in 92.4% (234 patients) (**figure 1**).

***Table 1** - Demographic characteristics of patients and quality of life questionnaire results for the treatment given (scale from 0 to 5).*

Characteristics		
Patients, n (%)	256 (100%)	
Sex, n (%)		
Women	133 (51.8%)	
Men	123 (48.2%)	
Age, years, mean (SD)	27 (13.8)	
Diagnosis of allergic rhinitis, n (%)	246 (96.5%)	
Rhinitis classification		
Mild intermittent	31 (13.7)	
Moderate / severe intermittent	127 (56.2)	
Persistent	29 (12.8)	
Moderate / severe persistent	39 (17.3)	
Diagnosis of allergic bronchial asthma, n (%)	163 (63.9%)	
Bronchial asthma classification (total n: 148)		
Episodic / intermittent	128 (86.5)	
Persistent	20 (13.5)	
SCIT Composition, n (%)		
Grasses	144 (56.5%)	
Grasses + Olea	65 (25.5%)	
Olea	29 (11.4%)	
Others	17 (6.8%)	
Patients' satisfaction with the treatment based on asthma and rhinitis diagnosis	Moderate / Not satisfied (%)	Satisfied (%)
Rhinitis	33.3	35.5
Asthma	66.7	64.5
Total	100	100
Patients' satisfaction with the treatment given (scale 0-5)	General Population[1] Mean (SD)	
The healthcare professional who administers	4.3 (0.7)	
Improvement of symptoms	4.1 (0.7)	
The frequency (number of visits to be treated)	3.3 (1.1)	
Displacement (impact on daily life)	3.6 (1.2)	
Administration by puncture	3.2 (1.1)	
Physical discomfort following jab	3.1 (1.2)	
Overall satisfaction with treatment	4.1 (0.8)	

SD: standard deviation.
[1]Results of the questionnaire of patient satisfaction with the treatment given by satisfaction scale of 0 (very unsatisfied) to 5 (very satisfied).

***Figure 1** - Change in perceived effectiveness by patients between pre-treatment and post-treatment pollen season. Bars represent the individual improvement for each patient and the two vertical lines reflect the values in the pre-treatment and post-treatment pollen season, (p < 0.001). SD: Standard deviation.*

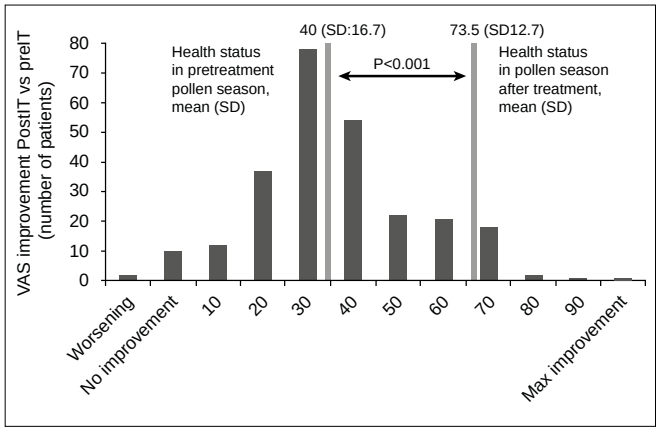
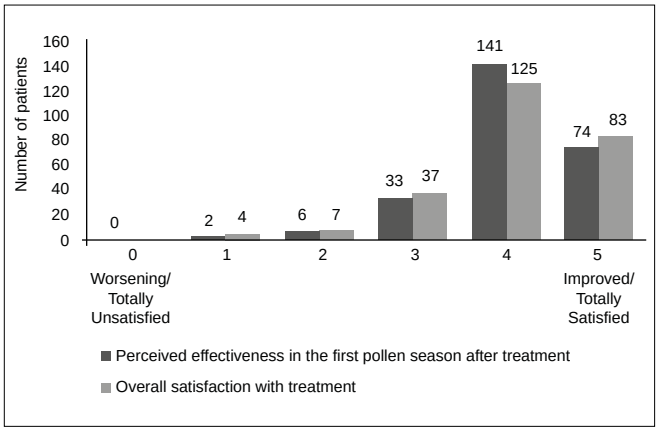


***Figure 2** - Perceived effectiveness in the first pollen season after treatment, and overall satisfaction with treatment.*
84% (215 patients) reported satisfaction or totally satisfied with improvement of symptoms. Pearson test 0.49; p < 0.001.



Satisfaction questionnaire

Overall patients' satisfaction with Allergovit® was close to very satisfied (mean 4.1; SD 0.8); 32.5% (83 patients) were totally satisfied (score 5) with Allergovit® and 48.8% (125 patients) reported a high degree of satisfaction (score 4) (**figure 2**).
In terms of degree of satisfaction, the most highly-rated aspect was the administration of the treatment by a healthcare professional, with 252 patients (98.4%) rating this positively, followed by the perceived clinical improvement of symptoms after having the treatment compared to before (mean 4.1; SD 0.7) (**table 1**).
According to **figure 2**, clear clinical improvement was reported by 84% of the patients (215 patients), with a positive correlation found between this variable and "Improvement of my allergy symptoms with this treatment" scale, using Likert scale to evaluate the correlation, taking into account the difference between the health status before and after treatment with Allergovit® (Pearson's Correlation: 0.49; p < 0.001).
There was also a positive correlation of satisfaction with the treatment's degree of effectiveness (coefficient of correlation: 0.34, p < 0.001).
The most critical aspects for the patients were those relating to discomfort following subcutaneous administration (mean 3.2; SD 1.1) and the associated to local adverse reactions (mean 3.1; SD 1.2).
However, in terms of the repercussions on daily activities of having to make the trip, 158 patients (61.7%) stated that it was no trouble at all (Score 4 and 5) and 61 patients (23.85%) said that it was not an issue (Score 3).

The treatment was well tolerated by most of the patients included in the study (254 patients, 99.2% of population). Only 2 patients reported to suffer "adverse events problems / intolerance", and that was the reason of treatment discontinuation. Due to this number of patients (n = 2), no specific exploratory analysis was developed.

5. Discussion

There are some randomised clinical trials (RCT) confirming the effectiveness and safety of SCIT with Allergovit® (6,7,18,20). In particular, it is worth mentioning the published studies that demonstrate the sustained long-term effectiveness of Allergovit® grass pollen in children, even for up to 12 years after having stopped the treatment (21,22).
Our study is the first Spanish study that tried to assess, under routine clinical practice conditions, the effectiveness, satisfaction and tolerability perceived by the patients, while also tried to confirm the effectiveness and safety data previously obtained in clinical trials conducted with this same high-dose hypoallergenic extract of pollen allergens.
Despite the limitations inherent to retrospective observational studies, they produce data on effectiveness and different variables in the context of routine clinical practice, providing useful and important information to supplement the RCT. With this in mind, The Brussels Declaration on Asthma recently announced the need for pragmatic studies that collect evidence from routine clinical practice (23).
One limitation of this study is in relation to the bias of memory the influence of other disorders which might affect all of the

questions, particularly those about general health before and after the treatment. In an attempt to minimise the influence of this limitation, the analysis of effectiveness focused on the responses to the question on improvement or worsening of the allergic symptoms after the treatment.

In this project, the short-term effectiveness observed after SCIT with Allergovit® in various clinical trials (6,7,8,17) could be aligned with what was observed from the perspective of the patients, since there was an improvement of 33.5 points on the visual analogue scale (from 0 to 100 points). We could therefore hypothesize that the effectiveness of the treatment was perceived after administration of Allergovit®, since the health of the patients improved significantly ($p < 0.001$). In terms of improvement as perceived effectiveness by the patient (at least a 4 on the 0 to 5 scale), the treatment was effective in 4 out of every 5 patients. Therefore, it should be emphasised that a high proportion of patients (over 96%) in the trial on hand reported to be very satisfied with the treatment, which could be related to increasing the adherence to SCIT, which in turn is a precondition for the aforementioned effects during and after SCIT.

More specifically, the aspect which, for the patient, represented greater satisfaction with the treatment was the fact that the subcutaneous injection was administered by a healthcare professional. The fact that patients have to attend their medical centre periodically to have Allergovit® administered, far from being an inconvenience, could mean that they are better monitored and have better control and follow-up of their condition by a specialist, and this in turn could encourage adherence to treatment. A recently published study demonstrated that adherence to treatment is significantly higher, and in line with patients treated with conventional immunotherapy, when the patient is monitored quarterly and not annually as in usual routine practice with patients who are prescribed AIT (4).

In this study, the effectiveness and tolerance results expected by patients could be confirmed. Moreover, patients treated with Allergovit® reported to be satisfied with the treatment, while the route of administration could not represent any inconvenience in terms of the desired adherence to treatment. The general level of patient satisfaction was positively associated with the improvement in their health after receiving SCIT with Allergovit®, and this was even greater when it was administered by specialist healthcare personnel.

VAS improvement could be comparable to that found in the post-marketing surveillance study by Hoheisel *et al.* (17).

In conclusion, the results obtained in this study show that subcutaneous immunotherapy with high-dose hypoallergenic pollen preparations could be effective and well-tolerated in "real-life" practice. The patients' conditions improved noticeably, with an effect in the first pollen season after starting treatment.

Appendix

Questionnaire to collect the patient satisfaction

Each of the following questions was answered by a Likert scale, with 0 representing strongly unsatisfied and 5 totally satisfied.

1. Which is your current satisfaction if the drug administration is done by a health professional?
2. Which is your current satisfaction if the drug administration is done by an injection?
3. Which is your current satisfaction with the treatment administration frequency (number of medical visit needed for the injection)?
4. My allergic symptoms have been improved or are worst with this treatment.
5. Which is your current satisfaction with the physical discomfort after the injection?
6. Choose from 0 (a lot) to 5 (nothing) the impact on your daily activities (quality of life, the need to travel, to receive the treatment, etc.)

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Hyper IgM Syndrome with low IgM and thrombocytosis: an unusual case of Immunodeficiency

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Key words
hyper IgM; CD40L

Summary
We report a 5 years old male child with low serum IgG, IgA and IgM levels, who presented with recurrent perianal and oral ulcers, intermittent fever, and protracted diarrhea. Despite the lack of typical respiratory symptoms, low serum IgM level and persistent thrombocytosis, an X-linked hyper-IgM syndrome (X-HIGM) was considered. Laboratory investigations revealed a diagnosis of hyper-IgM syndrome caused by CD40L deficiency.

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The X-linked hyper-immunoglobulin M syndrome (XHIGM) is a rare form of primary immunodeficiency, characterized by hypogammaglobulinemia and impaired cellular immunity. It is caused by several mutations of the CD40 ligand (CD40L), expressed on activated T lymphocytes, resulting in an inability to signal B-cells to undergo isotype switching (1), resulting in markedly reduced levels of IgG, IgA, and IgE with normal or elevated levels of IgM (2).

Male children with CD40L deficiency become symptomatic during early childhood, with recurrent pyogenic infections caused by capsulated or encapsulated bacteria, but they are also more prone to infections with intracellular pathogens such as *Pneumocystis carinii*, *Cryptosporidium parvum,* and *Leishmania.* Other clinical associations may include gastrointestinal ulcers, recurrent or protracted diarrhea, hepatitis, sclerosing cholangitis, neutropenia, thrombocytopenia, and anemia.

Diagnostic criteria (3) used for the US XHIGM registry, the Pan-American Group for Immunodeficiency, and the European Society for Immunodeficiencies, consist of two of the following: 1) mutation of CD40L; 2) a positive family history of a lateral male relative with XHIGM syndrome; and 3) defective expression of CD40L on activated T-lymphocytes. Here we report a case of a 5-year-old boy with defective expression of CD40L with pan-hypogammaglobulinemia including low-normal levels of serum IgM.

A 5-year-old North African boy presented to us with 3 days' history of fever, perianal and oral ulcers, and abdominal pain. His history was significant for multiple admissions due to recurrent perianal and oral ulcers, intermittent fever, and protracted diarrhea. A review of available laboratory data revealed persistent neutropenia and hypogammaglobulinemia, including low levels of IgM. The child was initially diagnosed as a case of congenital neutropenia, Kostman syndrome and hypogammaglobulinemia.

Table 1 *- Summary of immunoglobulin levels (mg/100 ml).*

Immunoglobulin class	Patient levels	Normal range
IgG	230-340	542-1515
IgG1	130-286	380-840
IgG2	69-109	83-543
IgG3	0.6-60	10- 92
IgG4	0.10-0.5	1-11
IgM (obtained 15 times)	29-37 (Except on two occasions when it was 40 and 44 mg/100 ml respectively)	40-230
IgA	45-90	48-301
IgE	< 2 u/l	1.6-30 u/l

Table 2 *- Flow cytometry results.*

Cell type	%	Absolute value
Lymphocytes	44-75%	3894-7300 mm3
CD4+ cells	36-50%	1437-2577 mm3
CD8+cells	14-35%	239-1579 mm3
CD56&CD16 cells	1-7%	1674-244 mm3
MHC Class 1	100%	3939-7300 mm3
MHC class2	20-37%	858-2044 mm3
CD 19+ CD40 cells CD40 L Status	Intact Lack of expression x 3	

A lack of satisfactory clinical response led his parents to contact our tertiary care center. Family history revealed the death of a male sibling at the age of 6 years from severe pneumonia. A review of the brother's medical records revealed hypogammaglobulinemia, albeit, with elevated levels of IgM (2,320 mg/l). The parents were healthy, as were two sisters. Our index patient had multiple admissions due to recurrent oral and severe perianal ulcers, perianal abscess, fever, and protracted diarrhea. The child also had persistent neutropenia and thrombocytosis.

Physical examination, performed during multiple admissions, revealed a similar clinical picture with severe oral and perianal ulcers, hepatosplenomegaly, and abdominal tenderness.

Summary of immunoglobulin levels and flow cytometry results are shown in the **tables 1** and **2**, along with normal ranges reported for pediatric subjects in his age range.

Quantitative analysis of immunoglobulins in serum was low, including IgM (except on two occasions, when it was found to be within normal limits). Flow cytometry results revealed intact CD19 + CD40 cells; however, there was a consistent lack of expression of CD154 (CD40 ligand) on the surface of activated CD4 + lymphocytes. This was repeated three times with similar results. Absolute (ABS) and relative numbers of lymphocyte subsets were normal, excluding the typical cases of severe combined immunodeficiency.

Antibody response to protein and polysaccharide antigens was impaired. Results of lymphocyte stimulation test / blastogenesis revealed a moderate depressed response to mitogens. Levels of complement components measured were elevated: complement C2 was 29.43 mg/l (normal = 4-24 mg/l) and complement CH50 was 662 U/ml (normal = 345-485 g/l).

Other laboratory investigations included: neutropenia, absolute neutrophil count = 0.20-9.2 × 10^9/L (normal = 1.37-7.50 x 10^9) and thrombocytosis, platelet count done multiple times with range of 650-1,344 × 10^9/L (normal = 155-435 x 10^9/L). The platelet counts continued to be elevated despite clinical improvement and normal Erythrocyte Sedimentation rate (ESR) readings between the acute illnesses. Hepatic profile included normal alanine amino transferase 13-35 U/L (normal = 10-35 U/L), aspartate amino transferase 25-27 U/L (normal = 10-45 U/L), and gamma glutamyl transferase 21-27 U/L (normal = 11-49 U/L).

All blood and urine culture results were negative. Peritoneal fluid culture showed many mixed organisms (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*, and *Proteus* species). Stool was positive for *Clostridium difficile* toxin during one admission, and stool culture yielded *Salmonella* group D.

Due to lack of an appropriate genetic testing facility in that country, we were unable to obtain mutation analysis on this patient. However, patient was diagnosed with hyper-IgM syndrome due to presence of 2 out of 3 criteria required for the diagnosis of XHIGM (3).

He was continued on intravenous gamma globulin and granulocyte colony stimulating factor and referred to our bone marrow transplant team for possible transplant as a curative therapy.

Although, low IgM in hyper IgM syndrome has been described previously (4). Our index case however, showed deviation from a typical case of hyper-IgM syndrome in the following domains: 1) There has been a consistent low level of IgM except on two occasions (19 out of 21 times). 2) There is a direct correlation

between the platelet count and the concentrations of plasma CD40L (5), a possible explanation of thrombocytopenia in XHIGM. Contrary to expectations, our reported case exhibited persistent thrombocytosis. Iron deficiency, which usually causes thrombocytosis, was ruled out. Extensive review of the literature failed to find any other published report of thrombocytosis in the context of hyper-IgM syndrome / CD40 deficiency. To our knowledge, this is the first case report of CD40L deficiency with low IgM and
thrombocytosis.

3) Lack of respiratory symptoms. *Pneumocystis jirovecii* pneumonia (PCP) is usually the first clinical evidence noted in 59% of XHIGM during early infancy (2). An important consequence of hyper-IgM syndrome is the susceptibility to recurrent infections including *Pneumocystis jirovecii* (43%) (6). Typically, these patients present with respiratory symptoms and pneumonia about 81% of the time (2). However, our patient showed a deviation from this typical clinical pattern.

Conclusions and clinical implication

An important clinical implication is that a diagnosis of hyper IgM syndrome may be missed or delayed in the context of low IgM levels and high platelet counts. This was true in our case, in which a workup for hyper-IgM syndrome was not performed until the age of 4-years because of low IgM levels and thrombocytosis.

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Early onset steroid induced posterior subcapsular cataract in a patient with common variable immunodeficiency: case reports and review of literature

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Key words

common variable immunodeficiency; glucocorticoid; posterior subcapsular cataract; glutathione reductase gene mutations

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Summary

Purpose. *To report early onset steroid induced posterior subcapsular cataract in a case of common variable immunodeficiency.* ***Methods.*** *Case report.* ***Results.*** *Here we report a 14-year-old male of steroid induced bilateral posterior subcapsular cataract in a common variable immunodeficiency patient with damaging mutations in Glutathione reductase gene, leading to hypersensitivity of patient to glucocorticoid (GC) products.* ***Conclusions.*** *In order to reduce the ocular side effects of the GCs there are some advisements, including a complete history, regular examination, GC should be prescribed in minimal dosage and minimal course, and as possible GC-sparing drugs should always be considered.*

Introduction

Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency disease (1-3). CVID is characterized by reduced serum immunoglobulin levels and increased susceptibility to recurrent bacterial infections, autoimmunity and malignancies (4-6). CVID may present at any age but the peaks of presentation are in childhood and early adulthood (1,7-9). The range of clinical manifestations is broad. Recurrent bacterial infections are most in respiratory and gastrointestinal tracts (3). Most frequent autoimmune disorders are idiopathic thrombocytopenia and autoimmune hemolytic anemia, and most common malignancies are gastric adenocarcinoma or lymphoma (3,10). Ocular manifestations in CVID patients are due to infections and usually present with conjunctivitis (11). There are a few unusual reports in the literatures, which are unilateral diffuse placoid choroidopathy (12), uveitis (13-16), optic disc neovascularization (16), retinitis (17), loss of retinal function (18), retinal vasculitis (19), unilateral peri-orbital redness, pain, proptosis and restriction of ocular movements (20), corneal perforation (21), bilateral optic neuritis (22), choroidal granulomas (23). Age independent cataract has not been reported as an ocular manifestation in a CVID patient so far. Our report is the first case report of bilateral posterior subcapsular cataract in a CVID patient with heterozygous mutations in glutathione reductase (GSR) gene leading to hypersensitivity to glucocorticoid (GC).

Case presentation

The patient is a 14-year-old male with a history of omphalitis at 12 days of age and recurrent post vaccination fever as his first complaints. He is a child from related parents (first cousin), with no family history of immunodeficiency. He experienced recurrent episodes of infectious diarrhea and upper and lower respiratory tract infections such as pneumonia, otitis media and sinusitis, which required recurrent hospitalization. He had scalp and scrotum abscess at 9 and 15 months of age. At the age of 1.5 years he was diagnosed with CVID based on panhypogammaglobulinemia, defective specific antibody production and normal B cells (**table 1**). He received regular intravenous immunoglobulin (IVIG), which resulted in serum immunoglobulin levels increase and improvement of clinical condition. At the age of 5 y, he presented with elevated liver enzymes. Viral hepatitis was excluded by negative results of HBS antigen, HCV antigen, PCR HCV and HBS antibody level of 76 IU/L. Liver biopsy was performed and chronic autoimmune hepatitis was diagnosed, and required prednisolone administration. During GC therapy, the patient suffered from progressive visual impairment and finally bilateral lens opacity was diagnosed at age 12 y in routine ophthalmic examinations. Visual acuity was diminished to 6/10 for both eyes, and his eye examination revealed abnormal Bruckner test (red eye reflex). He had no leukocoria, photophobia, abnormal extraocular movements, strabismus or nystagmus. Relative afferent pupillary defect (marcus gunn pupil) was negative for both eyes. Intraocular pressure was 16 mmHg in both eyes, with its normal range from 10 to 21 mmHg. Lids, conjunctives, corneas, anterior chambers and irises were all normal in examination. His drug history included IVIG 2.5-15 mg monthly since 12 years before (following diagnosis of CVID), prednisolone 10-50 mg daily since 8 years before (following diagnosis of autoimmune hepatitis), azathioprine, ursobil, colchicine, inderal and zinc plus. Our patient was candidate for cataract surgery and it was done successfully.

To investigate the genetic cause of hyper sensitive response of patient to steroid therapy, the next generation sequencing was performed using the method previously described, and the data obtained were filtered out for synonymous mutations and eliminated common variants, then prioritized the results for following genes: GILZ (GC-induced leucine zipper), SERPINE family (Serpin peptidase inhibitor), Cadherin family (cadherin-associated protein and E-cadherin), FGF2 (Fibroblast growth factor 2), IGF1 (Insulin-like growth factor 1), IGFBP family (Insulin-like growth factor binding protein) MAPK1,3 (Mitogen-activated protein kinase 1 and 3), CTFR (Cystic fibrosis transmembrane conductance regulator), PIK3 family (Phosphatidylinositol-4,5-bisphosphate 3-kinase), AKT family (akt murine thymoma viral oncogene), PTK2B (Protein tyrosine kinase 2 beta), ABC family (ATP-binding cassette), NFKB family (Nuclear factor of kappa light polypeptide gene enhancer in B-cells), REL family (v-rel avian reticuloendotheliosis viral oncogene), GSK family (Glycogen synthase kinase), IRS family (Insulin receptor substrate), RAS family (rat sarcoma viral oncogene), MAPK family (Mitogene activated protein kinase), GPX family (Glutathione peroxidase), FOXO family (Forkhead box) and WNT family (Wingless type MMTV integration site). We subsequently found 2 novel, heterozygote mutations in the *GSR* (Glutathione reductase) gene (**table 2**). This mutation was confirmed by means of sanger sequencing (**figure 1**). The mRNA accession number was NM_000637.3. The primers used were AGGAAGGGAGATCCAGAGGTT (ex10-F) and CCCTCACCAAGAAGGGAAGA (ex10-R), giving a product of 221 bp, as well as TGAAACTGTCAGAACTAGGGC (ex11-F) and GGGGAAAGAGGAAGGAAACCA (ex11-R), giving a product of 294 bp.

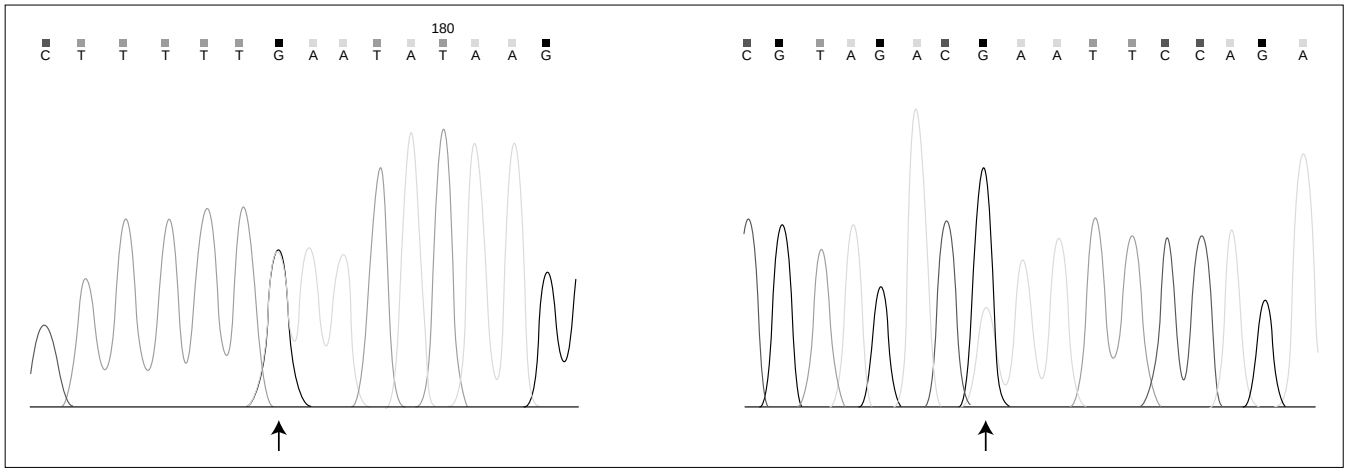
Table 1 *- Laboratory Data of the Patient with Steroid Induced Posterior Subcapsular Cataract at the Time of Diagnosis.*

Parameters	Patient	Normal Range
IgG (mg/dl)	150	650 - 1410
IgM (mg/dl)	18	55 - 210
IgA (mg/dl)	25	83 - 255
Anti-tetanus (IU/ml)	< 0.01	Upper than 0.1
Anti-diphtheria (IU/ml)	< 0.01	Upper than 0.1
White blood cell count (cells/µL)	4500	4500 - 13000
Lymphocytes (cells/µl)	1350	900 - 7800
CD3+ (cells/µl)	1160	495 - 2106
CD4+ (cells/µl)	669	243 - 4134
CD8+ (cells/µl)	445	171 - 2652
CD19+ (cells/µl)	94	36 - 2418

Table 2 *- Glutathione reductase gene mutation analysis of the CVID patient with steroid induced posterior subcapsular cataract.*

Gene	Nucleotide change	Protein change	Allele frequency	SIFT prediction	Type of mutation
GSR	c.G1081>A	p.E361K	Not reported	Damaging	Heterozygous
GSR	c.G1195>A	p.E399K	Not reported	Damaging	Heterozygous

Figure 1 *- Mutation analysis of GSR gene in a CVID patients presenting with early onset steroid induced posterior subcapsular cataract.*



Discussion

The range of CVID clinical spectrum is broad, but there are a few reports of ocular manifestations (as mentioned before) and no reports of cataract. Here we describe a CVID patient with bilateral cataract. Cataract is one of the major curable causes of blindness in children (24). Children cataract has a lot of etiologies, which are categorized in several major groups including ocular trauma, hereditary, disease-associated, ionizing radiation and glucocorticoids (25-29). Our patient had no ocular trauma and no ionizing radiation in his history. He was born with normal vision until 1 year before, so hereditary causes were excluded too. Numerous diseases are associated with cataract (29), but none of them was diagnosed in this patient, and the only systemic disease in our patient was CVID. CVID itself has not been reported as a cause of cataract so far. Our patient had a long history of receiving various drugs but the only drug that could cause his cataract was GC. In treatment of CVID, the GCs are mostly used to treat polyclonal lymphocytic infiltration, autoimmune disorders such as autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura, gastrointestinal problems for example enteropathy (3). Also, it has been used as the first line treatment for autoimmune cytopenia in these patients (30). Our patient had a lot of episodes of cytopenia plus autoimmune hepatitis, which led to administration of GCs. Long term use of systemic GCs can cause various ophthalmic side effects including ocular hypertension, serous chorioretinopathy and cataract (26,31-33). Steroid-induced cataract follows this pattern: 1) It is posterior subcapsular, 2) Mostly affects both eyes and progresses slowly, 3) Children are more affected than adult (34), which are all compatible with our patient. Prednisone and prednisolone are the most common GCs used. This is because of higher GC effects comparing to mineralocorticoids (35). Our patient has been receiving systemic corticosteroid (prednisolone) 10-50 mg daily since 8 years before. Many broad retrospective studies had shown the usage of GCs for a long period, whether in low doses, is an independent factor to predict happening of the many GC side effects (36,37). In one study on patients taking low doses of GC (prednisone 7.5 mg daily), long term using (more than 90 days) was associated forming of bruising, acne, weight gain and cataract (38). Posterior subcapsular cataract is really affecting the vision of patient and needs more rapid surgical intervention comparing to other subgroups of cataract. Susceptibility of patients for cataract after long term treatment with GCs is different. The patient should use at least for 1 year more than 10 mg daily oral prednisone (or the same dose of other GC) (35,39). The patient presented above, received at least more than 10 mg prednisolone daily for 8 years. Although there is not yet a real safe dosage of GC to prevent cataract, in 2 clinical trials which were done for 2 years on low dose administration of systemic GC, (prednisone 7.5 mg daily), 4 of 273 cases got glaucoma. This rate in the control group was 0, but the appearance of cataract did not differ (40). The results of these studies cannot be generalized because of limited course of GC administration and dosage. Unfortunately, early cataract may not have any symptoms up to the late levels.

Oxidation of lens proteins is one of mechanisms which causes cataract, especially age related cataract. There are a few papers showing this mechanism responsible for steroid-induced cataract (41,42). Steroids affect cellular processes by steroid response elements (GRE) in the promoter region of specific genes (43,44) including glutathione reductase gene. Glutathione is oxidized (forming GSSG) as a key substrate in the intracellular antioxidant systems, such as glutathione peroxidase / reductase.

This system detoxifies H2O2 to water in lens epithelial cells. When glutathione is decreased, this system oxidizes other cell proteins which leads to cataract formation. There are several mechanisms causes GSH level diminish (45-48), among which is defect in glutathione reductase activity (49-51). GSR reduces oxidized glutathione (GSSG) to regenerate GSH. When GSR impair glutathione is decreased, and glutathione peroxidase / reductase system oxidizes other cell proteins. Our study revealed compound heterozygous mutations in *GSR* gene following steroid administration which subsequently leads to GSR activity impairment and cataract formation.

In order to reduce the ocular side effects of the GCs there are some advisements: most importantly a complete history and physical examination should be taken, to identify the risk factors of getting the side effects of GC. Second, regular examination of the eyes is recommended to be done more frequently. Third, GC should be prescribed in minimal dosage and minimal course. Forth, as possible GC-sparing drugs should always be considered e.g. using anti immunoglobulin E monoclonal antibody can reduce the exacerbation of asthma which needs systemic GC and improve quality life of the patients (35).

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Eur Ann Allergy Clin Immunol Vol 48, N 5, 202-204, 2016

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Clinical and radiological signs of ABPA associated with airways infection with Aspergillus in the absence of specific IgE

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Key words
Aspergillus; allergic bronchopulmonary aspergillosis; IgE

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Summary
Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity reaction to Aspergillus that mainly affects patients with asthma. For diagnosis, elevated serum IgE level are needed according to Greenberger and Patterson criteria. We report a case of 43 years-old woman who developed ABPA with productive cough, fever and radiological findings of multiple confluent areas of consolidation in both upper lobes. Laboratory tests showed elevated peripheral eosinophil counts (9.3 x 10³/ml). In bronchial washing A. galactomannans *and* A. Fumigatus *were isolated, although we found normal levels of serum IgE, and the absence of serum IgG and IgE antibodies to* Aspergillus *and* A. galactomannans. *In conclusion, clinical and radiological signs of ABPA can be associated with* Aspergillus *infection also in the absence of a specific serum antibody reaction.*

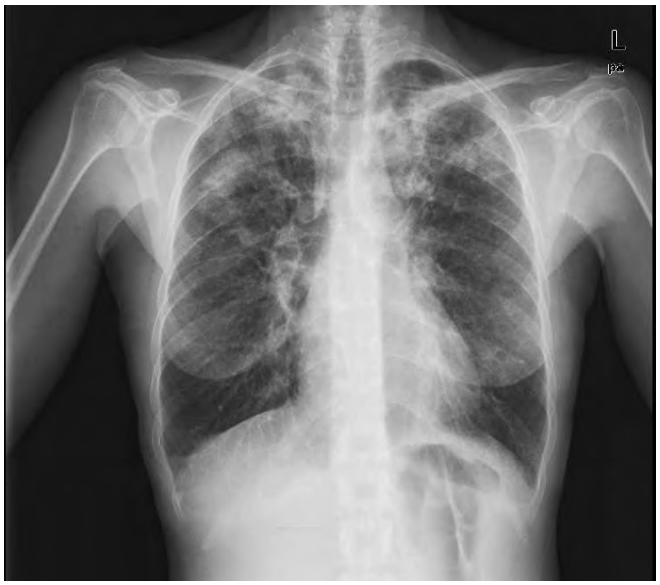
Introduction

Aspergillus, like other filamentous fungi, is primarily acquired from an inanimate reservoir, usually by the inhalation of airborne spore, leading to a variety of clinical syndromes, ranging from aspergilloma in patients with lung cavities, to chronic necrotizing aspergillosis in those who are mildly immunocompromised (1). Invasive pulmonary aspergillosis is a severe disease that is seen in immunocompromised patients, while allergic bronchopulmonary aspergillosis is a hypersensitivity reaction to *Aspergillus* antigens that mainly affects patients with severe asthma (1).

Case Report

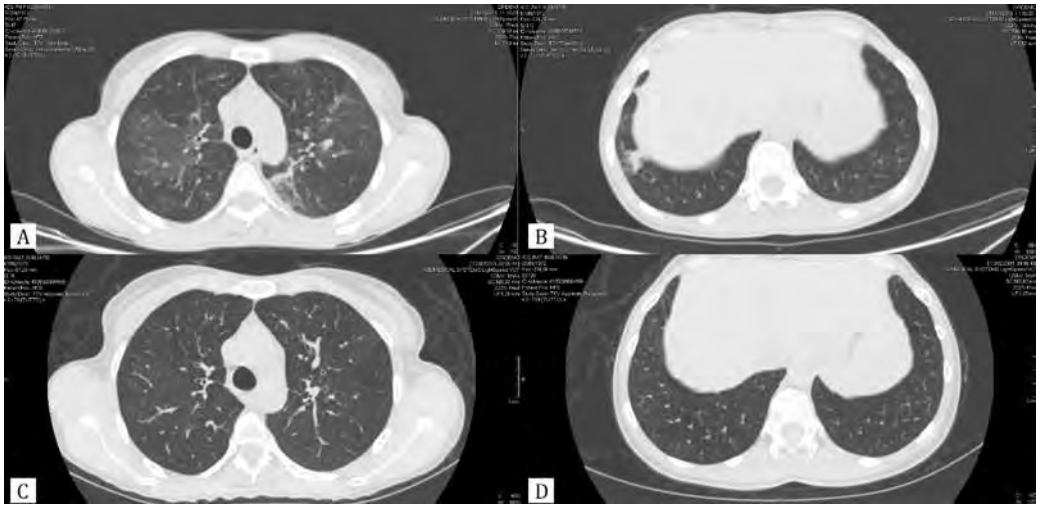
A 43 years-old non-smoker woman was referred to us for further evaluation of cough, dyspnea, fever, and peripheral pulmonary infiltrates. The patient had been well until about seven years before, when she started working in an open dusty environment. Subsequently, the patient experienced persistent rhinitis and several episodes of dyspnea. Lung function tests showed a modest obstructive ventilatory defect with a negative airway reversibility test; a chest X-ray (September 2012) was normal. The patient was diagnosed with asthma and allergic rhinitis and was treated with inhaled corticosteroids and bronchodilators, without benefits. Six months before admission, on August 2014, persistent cough and wheezing were developed. Laboratory tests showed serum IgE levels of 18 IU/ml (normal values, 0-180 IU/ml), elevated peripheral eosinophil counts (2.2 x 10^3/mm^3); a prick test for pollens and inhalants, including *Aspergillus* was negative; the pulmonary function test was slightly reduced, showing a forced vital capacity (FVC) of 1.95 L (63% of predicted value), forced expiratory volume in 1 sec (FEV1) of 1.27 L (48% of predicted value), and FEV1/FVC of 65.35 (81% of predicted value), 64.7 (80% of predicted value) after airway reversibility test. With-

Figure 1 *- Chest X-ray showed a parenchymal consolidation on the apex bilaterally and diffuse broncho-vascular markings of lungs.*



in six months, the symptoms remained stable up to one week before admission, when fever (> 38°C) developed with worsening cough and dyspnea. On admission, the patient complained of productive cough. On physical examination, she had coarse breathing sounds with crackles and wheezing in both lower lung fields. On laboratory test, the patient had hemoglobin of 12 g/dl, white blood cell counts 19.9 x 10^3/ml with 33.6% neutrophils, 15.4% lymphocytes, 46.9% eosinophils (9.3 x 10^3/ml) and 3.9% monocytes. On serum biochemistry, she had normal liver kidney and electrolyte profile, a reactive C protein of 25.76 mg/L (normal values, 0-3 mg/L), normal levels of neutrophilic cytoplasmic antibodies, angiotensin converting enzyme. Serum IgG antibodies to *Aspergillus* and serum *Aspergillus* galactomannans were absent. Total IgE levels were 11.3 UI/ml (normal values, 0-87 UI/ml) and specific IgE were absent. Arterial blood gas analysis on room air showed pH of 7.50, PaO_2 54 mmHg, $PaCO_2$ 30 mmHg, HCO_3- 23.4 mmol/L, and SaO_2 of 93.1%. On chest radiography, the patient had multiple confluent areas of consolidation in both upper lobes (**figure 1**). On chest CT scan the patient had ground glass opacities in both upper lobes and in right lower lobe (**figure 2a-b**). Neither pleural effusion nor bronchiectasis were present. A bronchoscopy was performed. This showed no macroscopic lesions, diffusely hyperemic mucosa and many yellowish tenacious secretions. On bronchial washing no malignant cells were observed. Bacterial culture test, *Mycobacterium Tuberculosis* complex and atypical mycobacteria polymerase chain reaction (PCR) were negative. At the microscopic examination, leukocytes, squamous epithelial cells and fungal hyphae were observed. The *Aspergillus* galactomannans from washing was positive, *A. fumigatus* was isolated from bronchial washing. Peripheral blood eosinophilia, pulmo-

Figure 2 *- Thorax-CT with contrast before treatment showed a diffuse pseudo-nodular interstitial thickening with ground-glass appearance, mainly present in the upper lobes bilaterally and right lower lobe (A-B). It was also possible to see a parenchymal consolidation in the left pulmonary apex and a small nodular lesion in the upper right lobe. No signs of pleural effusion or lymphadenopathies in the mediastinum area were present. After two weeks of therapy it has been possible to see a resolution of parenchymal consolidation and ground-glass areas reported in the previous CT (C-D).*



nary infiltrates and growth of *A. fumigatus* from bronchial washing suggested allergic bronchopulmonary aspergillosis (ABPA) (2). We started steroids at 1 mg/kg/day and voriconazole was added to her regimen. Two weeks later, the symptoms were improved. This was accompanied by the chest CT findings showing disappearance of the pulmonary infiltrates (**figure 2c-d**). Steroids were gradually tapered over a period of two months and the evolution was good.

Discussion

ABPA is a hypersensitivity reaction to *Aspergillus* antigens, mostly due to *A. Fumigatus*. It is typically seen in patients with long-standing asthma or cystic fibrosis. Greenberger and Patterson recently modified the diagnostic criteria for ABPA (3). Not all of these criteria need to be present to diagnose ABPA. The minimal criteria for diagnosis are asthma, immediate skin reactivity to *Aspergillus*, serum IgE level > 1,000 ng/mL, history of pulmonary infiltrates, and elevated levels of serum IgE and IgG antibodies to *A. Fumigatus* (3,4). Some aspects of this case are controversial and deserve to be addressed. First, ABPA is a syndrome seen in patients with severe obstructive lung disease, most commonly asthma (2). Our patient had only a mild obstructive lung defect developed after she started working in an open dusty environment, when she was possibly exposed to *Aspergillus* spores. Second, in atopic individuals, exposure to fungal antigens causes the formation of IgE antibodies directed at the antigen, re-exposure will then result in mast cell degranulation and eosinophilic infiltration (5). However, our patient had normal levels of total IgE, which suggested that she was not an atopic individual. Third, and more important, she did not have specific IgE or IgG. Specific IgE-mediated type I and specific IgG-mediated type III hypersensitivity reactions are proposed to play an important role in the immunopathogenesis of ABPA caused by *A. fumigatus* (6). Elevated aspergillus-specific IgE levels are a hallmark of ABPA. However, ABPA has been described in a child with cystic fibrosis and low serum IgE levels (7), and in an adult with concomitant common variable hypogammaglobulinemia (8). Thus, in unusual circumstances, the clinical and laboratory features of ABPA may be present in the absence of increased IgE.

Conclusions

In conclusion, clinical and radiological signs of ABPA can be associated with airways infection with *Aspergillus* also in the absence of a specific serum antibody reaction.

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Self-reported hair loss in patients with chronic spontaneous urticaria treated with omalizumab: an under-reported, transient side effect?

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Key words

chronic spontaneous urticaria; omalizumab; hair loss; side effect; quality of life

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Summary

Omalizumab has been recently approved for treating patients with refractory to H1- antihistamines chronic spontaneous urticaria (CSU). Although hair loss is listed among omalizumab side effects, there are no available data to estimate its frequency. We describe for the first time hair loss as a side effect associated with omalizumab administration in three women, 38, 62 and 70 years old, suffering from refractory to H1-antihistamines CSU. This information was retrieved from their Chronic Urticaria Quality of Life Questionnaires. Despite this side effect, all patients agreed to continue omalizumab regular administration. Hair loss appeared to be transient, lasting up to four months. All cases finally benefited from omalizumab continuation.

Introduction

Omalizumab is a recombinant humanized IgG1 monoclonal antibody that selectively binds to the receptor-binding portion of circulating, free immunoglobulin E (IgE) antibodies, regardless of its specificity. Omalizumab may suppress histamine-induced skin reactions, through the reduction of the number of the high-affinity receptors for the Fc region of IgE (FcεRI) on mast cells and basophils, which seem to downstream relevant cellular activation mechanisms (1).

Omalizumab is currently approved for treating adult and adolescent patients 12 years and older with severe or moderate to severe allergic asthma, in more than 90 countries including the US since 2003, and in the EU countries since 2005. In the EU, it has been additionally approved for the treatment of severe persistent allergic asthma in children 6 years old and above. Recently, in March 2014, the European Commission approved the use of omalizumab as an add-on therapy for the treatment of chronic spontaneous urticaria (CSU) in adult and adolescent (12 years and above) patients with inadequate response to H1-antihistamine treatment (2). The approved dose is 300 mg by subcutaneous injection every four weeks. This dose was concluded after evaluation of the efficacy and safety of omalizumab in three Phase III clinical trials: ASTERIA I (3), ASTERIA II (4), and GLACIAL (5) in nearly 1,000 CSU patients not responding to antihistamines.

In adult patients with allergic asthma, the most common side effects (seen in between 1-10%) associated with omalizumab include headache and injection site reactions (swelling, redness, pruritus and pain). Similarly, in patients with chronic spontaneous urticaria, the most common side effects, additionally to the aforementioned ones, include sinusitis, arthralgia and upper respiratory tract infection (2). In the Summary of Product Characteristics (SmPC) [available online in (2)], there are side

effects without enough available data to estimate their frequency. These include muscle pain, joint swelling and hair loss. We describe three patients with chronic spontaneous urticaria treated with omalizumab, all of which experienced hair loss after the first administration of 300 mg omalizumab.

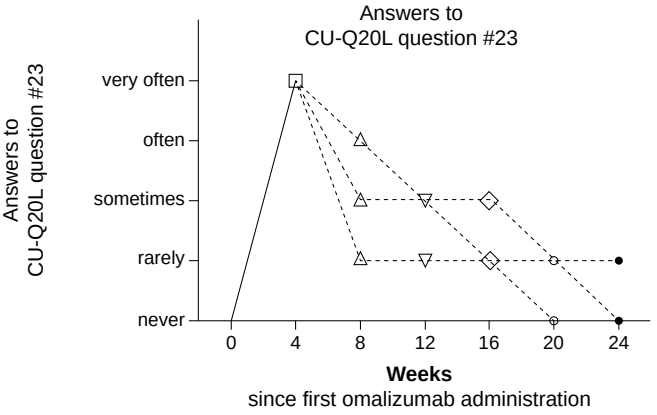
Case Series Presentation

Three women, 38, 62 and 70 years old suffering with chronic spontaneous urticaria (CSU) with angio-edema for 6, 5 and 3 months, respectively, were referred for further evaluation and treatment. All had CSU refractory to treatment with H1-antihistamines, with no other significant illnesses and no surgeries in the past apart from the oldest lady who was on 100 μg levothyroxine sodium daily the last 12 months due to Hashimoto's thyroiditis.

At the initial assessment their urticaria control tests (UCT) were 5, 6 and 6, respectively (6). None of them responded satisfactorily to a four-week course of a fourfold dose of levocetirizine (10 mg bid) (7) in addition to ranitidine 150 mg bid and montelukast 10 mg sid. During these four weeks, all of them required at least one 3-day course of methylprednisolone 8 mg bid due to angio-edema of the lips and/or eyelids and severe pruritus (8). All patients had positive autologous serum skin test (ASST) (9) and were offered the option to be treated with a course of cyclosporine. They all refused due to the described side effects, therefore omalizumab was prescribed. Urticaria activity scores (10) summed over a week (UAS7) at the day of first administration of omalizumab were 25, 27 and 30, respectively. All patients filled out the chronic urticaria quality of life questionnaire (CU-Q2oL) (11). In this questionnaire the last question "Do you suffer side-effects from the medications you take for hives?" was answered with "never" from all patients. Four weeks after the first administration of omalizumab, the UAS7 was slightly improved to 20, 21 and 25 respectively.

Interestingly, all of them changed their report about any side effects in the CU-Q2oL from "never" to "very often". This occurred on early November, mid-December and mid-April, respectively. Only in the oldest patient the reported hair loss was evident by scalp inspection. All patients were informed that hair loss might be a rare side effect of omalizumab and they were all referred for a dermatological examination which in all cases revealed Telogen Effluvium; however, all three agreed to continue omalizumab regular administration. The older lady was referred to her endocrinologist for reevaluation of her thyroiditis, which was found to be under control. Interestingly, this side effect was proven to be transient in all patients, with no need for any special relevant treatment, and to be correlated with urticaria improvement. The improvement course of the hair loss is presented in **Figure 1**. By week 20, all patients had UAS7 < 16 and UCT > 12. All patients are still on omalizumab. One patient still reports mild, but acceptable hair loss that does not seriously affect her quality of life.

Figure 1 *- Scores (0: , 1: , 2: , 3:) from the replies to the question number 23 of the CU-Q2oL questionnaire ("Do you suffer side-effects from the medications you take for hives?") the three patients with hair loss reported since the first administration of omalizumab (week "0").*



We describe for first time, to our knowledge, three cases of mild to moderate hair loss as a side effect associated with omalizumab administration. Since the approval of omalizumab for CSU, 80 patients (46 females) in total have been treated with omalizumab in our center. Although hair loss is listed among the side effects in the SmPC of omalizumab, there are no available data to estimate its frequency. Intriguingly, only the patient with the evident hair loss complained about this side effect, while the other two reported it only when they were in particular asked about their reply to the relevant question of the CU-Q2oL questionnaire. This highlights the importance of the CU-Q2oL questionnaire when assessing urticaria patients, and the fact that hair loss may potentially be underreported when it is not so severe.

All three patients characterized hair loss as a major, distressing side effect, significantly affecting their quality of life, even when there was no evident consequence of it. This side effect could not be attributed to a known trigger from the specialists; however it was still distressing, even as a self-reported side effect.

It is very difficult to pathophysiologically explain why this side effect occurs. It could be speculated that since mast cells appear to have some influence on hair cycle regulation (12), downregulation of mast cell releasability (13) by omalizumab may interfere with this regulation. However, it is interesting that this symptom was transient and it was associated in all cases with significant CSU improvement.

Conclusion

Hair loss, even as a self-reported side effect, might worsen the quality of life in patients with already deteriorated quality of life due to severe CSU. However, this symptom doesn't seem to be a good reason to stop treatment with omalizumab since it appears to be transient, lasting for 3 to 4 months, in patients who seems to finally benefit from continuous regular administration of omalizumab.

Abbreviations

CSU: chronic spontaneous urticaria
SmPC: Summary of Product Characteristics
UCT: urticaria control tests
UAS7: urticaria activity scores summed over a week
CU-Q2oL: chronic urticaria quality of life questionnaire

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