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1. **Summary and key words**: english, limited to 15 lines.
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For example:
Paupe J, Scheinman P (Eds.). Allergologie Pédiatrique. Flammarion, Paris, 1988: 324-42.

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- Figures always on separate numbered sheets and legends on the back in pencil
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Letter from the new Editors

From this issue of European Annals of Allergy and Clinical Immunology, Riccardo Asero steps down as Editor in Chief of the journal after five years of outstanding work. The editorial board and the new co-editors in chief all thank him for having placed European Annals of Allergy and Clinical Immunology among the most influential journals in the field of allergy and clinical immunology.

Readers' number has increased and metrics have improved over the years, with the support of the AAIITO (Associazione Allergologi Immunologi Italiani Territoriale e Ospedalieri) and SPAIC (Sociedade Portuguesa de Alergologia e Imunologia Clìnica) as well as of the editorial board and staff. Practical approach has become a peculiarity of original papers, case reports and reviews published in the journal and therefore very much appreciated by clinicians and clinical researchers. Young as well as established scientists have increasingly considered European Annals of Allergy and Clinical Immunology for submitting their manuscripts and clinicians for sharing their case reports and clinical expertise.

Due to these reasons, citations have increased steadily and the journal is now in the Web of Science database and its ESCI (Emerging Sources Citation Index) will be under evaluation by Thomson Reuters in the next two years, the last step of the process leading to the Impact Factor. Also the SJR index trend confirms the impressive work done in the past five years (see Table 1).

As new co-editors in chief, our first goal is to continue Riccardo Asero's work, consolidating the reputation of the journal and improving its impact on the community of allergy and clinical immunology specialists.

We strongly believe in the unique feature of the journal, which in turn represents that of the scientific societies supporting it: a clinical and practical approach based on a sound scientific background. We therefore welcome old and new Authors to share their results and ideas with the colleagues through the pages of European Annals of Allergy and Clinical Immunology.

We are committed to making speedy decisions, to providing authors with high quality reviews and readers with an essential tool for both their daily practice and continuous education. We look forward to your contribution!

Table 1 *- Scientific influence and impact of an average article published on some allergy journals (2015 data)*

Journal	**SJR**
The Journal of Allergy and Clinical Immunology	5.513
Allergy	3.048
Clinical and Experimental Allergy	2.184
Annals of Allergy Asthma and Immunology	1.268
International Archives of Allergy and Immunology	1.164
Allergy and Asthma Proceedings	0.798
Asian Pacific Journal of Allergy and Immunology	0.560
Journal of Investigational Allergology and Clinical Immunology	0.552
European Annals of Allergy and Clinical Immunology	0.346 (0.106 in 2011)
Clinical and Experimental Allergy Reviews	0,291
Allergo Journal	0,183
European Journal of Inflammation	0,167
Japanese journal of clinical immunology	0,138
Revue Francaise d'Allergologie	0,136
Clinical and Translational Allergy	0,119
Revista Portuguesa de Imunoalergologia	0,112
Italian Journal of Allergy and Clinical Immunology	0,103
Review of Allergy and Clinical Immunology	0,101
Annali Italiani di Dermatologia Allergologica Clinica e Sperimentale	0,100

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Parietaria pollination duration: myth or fact?

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Key words
Parietaria; pollen; pollen count; Italy

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Summary
Background. *Even though the* Parietaria *pollen season may be rather long, it is commonly thought that* Parietaria *pollen is a perennial allergen present along the whole year.* ***Objective.*** *This study aimed at investigating the duration of* Parietaria *pollen season during a 10-year period in Italy, analysing also the annual pollen quantity and the differences among geographical areas.* ***Methods.*** *Pollen count was assessed daily for 10 years. Globally, ten Italian centers measured* Parietaria *pollen count. Start date, peak date, end date, duration (days), peak value, and seasonal pollen index were evaluated in each center.* ***Results.*** *Ten-year* Parietaria *pollen count demonstrates that the pollen season usually lasted for 6-7 months in Italy. There are important differences among centres, mainly attributable to geoclimatic factors.* ***Conclusion.*** *This study demonstrates that* Parietaria *pollen season lasts about 6-7 months with two peaks (mainly in spring and lower in autumn) in Italy with important geographical variations. This information may have clinical relevance in managing patients allergic to* Parietaria.

Introduction

Allergic rhinitis (AR) is characterized by an IgE-mediated inflammation. Pollens are the most common allergenic sources causing AR. Each pollen type has a specific pollination season and biological properties, mainly concerning its pro-inflammatory activity (1). In addition, it has been demonstrated that allergic inflammation and symptom occurrence are closely related to the duration of pollen exposure (2), thus giving to each specific pollen allergy peculiar clinical characteristics.

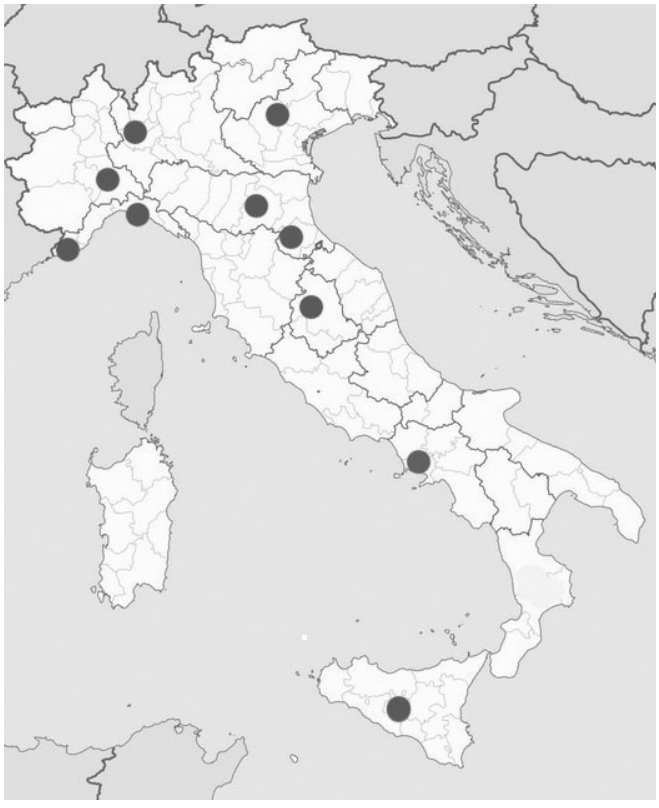
Parietaria is a widespread weed in the Mediterranean area, and many people are allergic to it (3). *Parietaria* belongs to the *Urticaceae* family; although many species exist, the most relevant are *Parietaria officinalis* and *judaica* concerning the AR pathogenesis. The term "parietaria" derives from the Latin word *paries* (wall), as it easily grows in the shade of old walls.
The pollen is small, the mean diameter of pollen grain of *Parietaria* is more about microns (more than PM10), and as consequence only a few grains can penetrate in trachea but they absolutely aren't able to reach terminal bronchioles. As a consequence, the high frequency of asthma is induced by bronchial

inflammation deriving from inflammatory events occurring in upper airways and other immunological mechanisms (probably also paucimicronic particles carrying allergens. Indeed, patients allergic to *Parietaria* frequently suffer also from asthma (4).
The *Parietaria* pollination season may be rather long, so that there is the popular belief that the symptoms for *Parietaria* allergy may be present actually along the whole year. This thought may have a practical implication also about allergen immunotherapy (AIT) prescription. In fact, many doctors prefer to prescribe AIT in *Parietaria* allergic patients using continuous courses, while they usually prescribe pre-co-seasonal AIT course for allergies to other pollens.
Though depending on climatic factors, the real duration of *Parietaria* pollen season is never perennial. Actually, *Parietaria* pollen season usually has two peaks: the main peak during spring and the second during early autumn. In autumn, there is a low peak in comparison to the very high spring concentration.
In this regard, two studies were recently published. The first study demonstrated that nasal allergic inflammation is closely associated with the duration of *Parietaria* pollen season, that lasted about 6 months in Bari, South of Italy (5). The second one confirmed the 6-months duration of *Parietaria* pollen season in Genoa and showed that a single pre-co-seasonal SLIT *Parietaria* course could be sufficient to reduce symptom severity and medication use (6).
As the previous studies were conducted in two defined geographical areas, the present study aimed to investigate the *Parietaria* pollination duration and quantity over a 10-year period in ten Italian centres located along the Italian peninsula.

Materials and Methods

We retrospectively analyzed the data concerning ten Italian centres: Bologna, Bordighera, Busto Arsizio, Caltanissetta, Città di Castello, Faenza, Genoa, Naples, Novi Ligure, and Verona (**figure 1**). The data concern a 10-year period (2004-2013).
The *Parietaria* pollens were assessed and analyzed according to validated methods (7-11). The pollen counts were recorded by a Hirst pollen trap (VPPS 2000, Lanzoni Srl, Bologna, Italy). The Hirst pollen trap was specifically designed for sampling pollen and fungus spores. Flow rate is fixed and provided by an external vacuum pump. The orifice of the spore trap (2-14 mm) was set 0.5 mm from the trapping surface (sticky tape). The airflow was 10 L/min, and the speed of the trapping surface was 2 mm/h. The apparatus always remained in the same place, 20 m above ground level and far from any pollution source, and it was permanently exposed to wind by means of a rotating air vane. The Hirst-type sample provides daily pollen trapping on sticky tape, which is transferred to microscope slides. Each slide is stained with fuchsin, and is read using an optical microscope at 250 magnification.
The reading of the slide takes place in a qualitative manner, defining the individual particles, and quantitative, by reading five longitudinal bands, using a statistical method. Data are expressed as average daily concentration considering the day from 0 to 24 (n/m3). The pollen count values, relative to the scanned surface, are extrapolated to the entire surface of sampling.
We considered the following parameters: Timing of pollen season, by investigating Start dates, Peak dates, End dates (these data were converted to the day of the year from 1 January), Duration of pollen seasons (days), Peak value (the highest daily pollen concentration, grain/m3), Intensity of pollen season (total amount of pollen during) by describing the seasonal pollen index (SPI). The period from which the sum of daily mean pollen concentrations reaches 1% of the total sum corresponds to start of pollen season, the time when the sum reaches 99% of the whole pollen amount corresponds to the end of pollen season (11).
The data were expressed as the mean of 10 consecutive years: from 2004 to 2013, considering all centers. Data analysis was performed with the GraphPad software package analysis (GraphPad Prism Software Inc, San Diego, USA).

Figure 1 *- Map of Italy with the centers that participated in the study.*



Results

Globally, there was a relevant difference among centers on the considered parameters. The Bordighera center shows the most precocious start and the latest end of pollen season, hence the longest duration (**table 1** and **figure 2**). *Parietaria* pollen season starts at the end of February and ends at the end of October. We considered the cut-off of 20 pollens/mc as the concentration able to to induce symptoms in *Parietaria* allergic subjects (12).
In Northern Italy, namely in Bologna, Bordighera, Busto Arsizio, Faenza, Genoa, Novi Ligure, and Verona, *Parietaria* season started at early April and ended in August, with two evident peaks: the most relevant in spring and the other during late summer. In Central Italy (Città di Castello), the *Parietaria* season started in early April and ended at the end of October, with a peak in May-June and another in September. In Southern Italy (Caltanissetta and Naples), the *Parietaria* season started in February and ended in August-September, with a peak between April and May.

Table 1 *- Considered Parietaria parameters for ten Italian centers.*

Center	Descriptive statistic	Start date	Peak date	End date	Duration (days)	Peak value (P/m3)	SPI (pollen)
Bologna	Max	127	175	295	191	244	10423
	Min	106	105	284	157	71	5264
	Mean	104	142	290	181	172	7115
Bordighera	Max	47	145	351	320	1050	16283
	Min	17	90	325	290	222	13677
	Mean	31	106	337	308	507	14980
Busto Arsizio	Max	122	153	318	286	880	25304
	Min	32	92	278	157	121	8263
	Mean	88	119	293	205	405	16724
Naples	Max	63	108	294	287	297	10199
	Min	15	92	231	189	63	2330
	Mean	41	104	272	231	142	6952
Caltanissetta	Max	50	144	333	289	890	20740
	Min	21	91	282	232	255	7209
	Mean	41	111	305	264	551	12697
Città di Castello	Max	118	127	350	200	1200	16338
	Min	31	56	260	142	245	8801
	Mean	87	101	288	170	478	12877
Faenza	Max	128	96	310	202	161	10282
	Min	89	49	279	151	106	5457
	Mean	108	75	295	175	134	7787
Genoa	Max	106	317	355	340	1050	6370
	Min	15	89	285	178	91	2701
	Mean	72	150	307	235	291	4305
Novi Ligure	Max	121	181	301	195	222	17234
	Min	106	138	290	160	97	6762
	Mean	113	160	295	182	190	12540
Verona	Max	104	206	350	280	1729	22213
	Min	70	87	265	165	208	12016
	Mean	89	114	294	205	1047	16925

Figure 2 - *Parietaria pollen count: start date, end date and duration of pollen seasons during the 2004-2013 period in Italy, in the nine centers considered.*

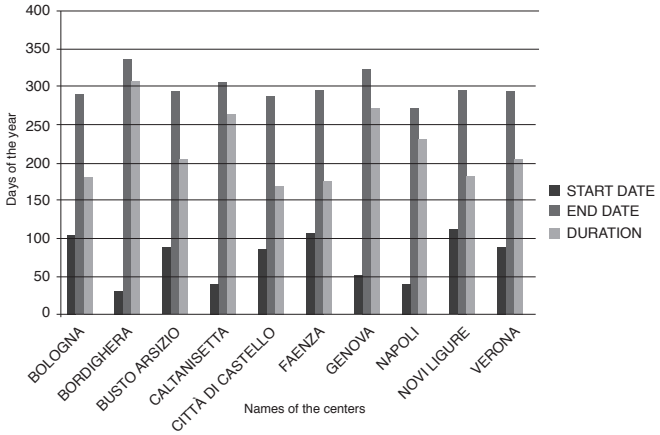
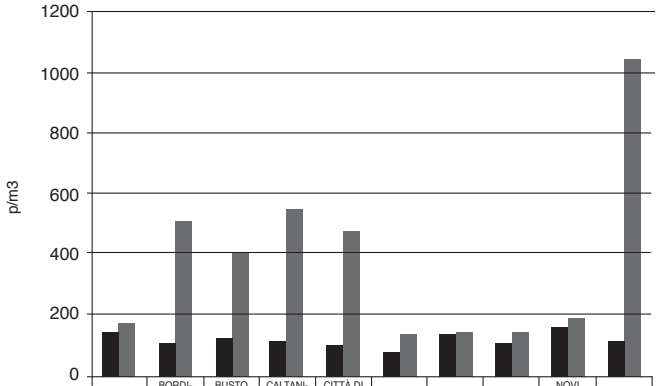
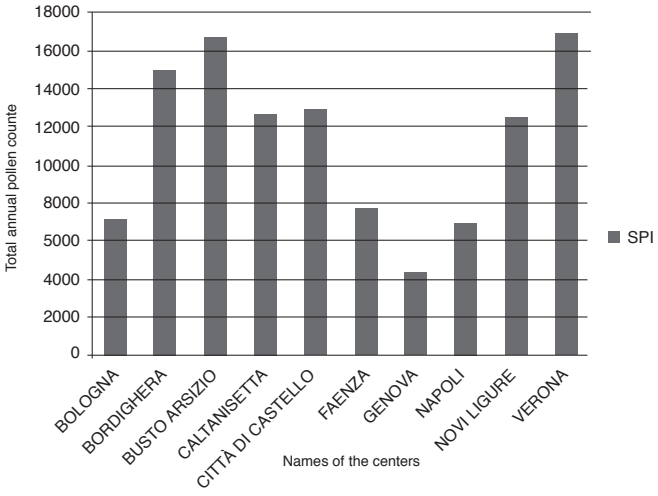


Figure 3 - *Parietaria pollen count: peak date and peak value, during the 2004-2013 period in Italy, in the nine centers considered.*



	BOLOGNA	BORDI-GHERA	BUSTO ARSIZIO	CALTANI-SETTA	CITTÀ DI CASTELLO	FAENZA	GENOVA	NAPOLI	NOVI LIGURE	VERONA
PEAK DATE	142	106	119	111	101	75	134	104	160	114
PEAK VALUE	172	507	405	551	478	134	140	143	190	1047

Figure 4 - *Parietaria pollen count: total amount of pollen during pollen season (seasonal pollen index: SPI) during the 2004-2013 period in Italy, in the nine centers considered.*



Discussion

Pollen allergy, such as hay fever, is the most common allergic disorder, as it affects up to 25% of general population (3). In this regard, *Parietaria* allergy is very frequent mainly in the Mediterranean area, as about fifty per cent of allergic patients are sensitized to it (13).

Many physicians believe that *Parietaria* pollen season may hold nearly over the whole year; this conviction may have a consequence in the clinical practice, for example on the schedule of Allergen Immunotherapy (AIT).

Previously, it has been reported that allergic inflammation and symptoms are closely related to *Parietaria* season's duration, which lasted about 6 months, in two different Italian geographic areas, such as in Liguria and Apulia (5,6). Moreover, a recent study over a 30-year period demonstrated that *Parietaria* pollen concentration tended to increase over time. This fact may explain the consistent increase in patients sensitized to *Parietaria*.

The present study addressed the assessment of the real duration of *Parietaria* pollen season in Italy, considering a 10-year observation period. The findings show that *Parietaria* pollination lasts 6-7 months on average with two main peaks: the most important during mid-spring and a lower peak during early fall, but with remarkable differences among geographic areas. The differences are obviously dependent on the climate characteristics of each region.

The present outcomes are substantially consistent with previous surveys. A 5-year aerobiological study, conducted in 1984-1988, evidenced that *Parietaria* allergy is a relevant issue in Italy, differing from most of European countries (14). Another study demonstrated that *Parietaria* season trended to prolong and its pollen count trended to increase over time (15).

The main limitations of this study is that it was conducted only on aerobiological data without clinical parameters, and that the considered period was relatively short. On the other hand, the strength of this study is the relevance of well-balanced distribution of participant centres.

Conclusions

This study demonstrates that *Parietaria* pollen season lasts about 6-7 months in Italy, with important geographical variations. This information has clinical relevance in managing patients allergic to *Parietaria*.

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Immediate adverse reactions to intravenous immunoglobulin in children: a single center experience

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Key words
Intravenous immunoglobulin; adverse events

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Summary
Intravenous immunoglobulin (IVIG) is commonly used in primary and secondary immunodeficiency diseases as well as autoimmune conditions as immunomodulatator treatment. Immediate adverse events which are generally mild and occur during infusion are seen in 6 hours. Reported immediate adverse events are in a wide range from 1%-40% in pediatric patients. 115 patients who received IVIG (except newborns) were included into this crosssectional study. IVIG was given to patients for primary immunodeficiencies (n=8), ITP (n=65), Kawasaki disease (n=11), secondary immunosupression (n=28), and passive immunization (n=3). 5%, 10% IVIG preparations and pentaglobin were used. Headache, fever, chills, nausea, rash, arthralgia, myalgia and back pain were accepted as mild immediate events. There were 62 (54%) boys and 53 (46%) girls aged 1 month-18 years. Mean age of the group was 7.4±4.6 years. Immediate adverse events due to IVIG infusions were seen in 29 (25.2%) of all patients. Gender and types of the disease were not different in significance regarding the presence of adverse events. The rate of adverse events did not change with receiving pre-medication. The most common reaction was fever/chills. Immediate reactions were seen in first 6 hours in 7 patients and during infusion in the remaining. They were treated with slowing of the infusion rate and infusion was stopped in 3 patients because of moderate events. Because of the increasingly use of IVIG therapy, it is important to know the side effects. High doses, high infusion rates, accompanying infection may worsen the adverse effects especially in primary immunodeficiency diseases.

Introduction

Intravenous immunoglobulin (IVIG) has been widely used for treatment since 1981 (1). Its area of usage is widening such as prophylaxis and replacement therapy for primary and secondary immunodeficiencies and for immunomodulatory effects in autoimmune and inflammatory conditions such as immune trombocytopenic purpura (ITP), Kawasaki disease, and Guillain-Barré syndrome (2,3).
IVIG is a biologic product prepared from donor serum pool after a series of procedures such as ethanol fractionation and adding stabilizers (4). Although these steps remove immunoglobulin aggregates, adverse events are seen frequently (5). Adverse reactions with IVIG infusions vary from 1% to 81%, but mostly about a rate of 20% (3,6). These events can be classified as immediate, delayed and late adverse effects. Immediate adverse events such as fever, headache, arthralgia, flushing, rash, acute renal failure, anaphylaxis, hemolysis due to IVIG are seen in the first 6 hours of the infusion, delayed adverse events develop in 72 hours to 1 week and late events can be seen in weeks to months after IVIG administration. Reported immediate adverse events are in a wide range from 1-40% in pediatric patients (7-11). These reactions are generally mild and occur during infusion (12).
IVIG preparations differ in their composition and properties, and these factors can affect the efficacy and tolerability of IVIG. They are prepared at various concentrations with 5%, 10% and 3-12% (8).
In the present study, we aimed to evaluate the frequency of immediate adverse reactions due to IVIG infusions in patients received IVIG mostly due to autoimmune diseases.

Materials and Methods

This study was conducted between January 2009 and December 2009 in a tertiary referral center in Istanbul. One hundred and fifteen patients who received IVIG in pediatric clinics (except newborns) were included, and a cross-sectional study was performed. In patients who were receiving IVIG infusions on a regular basis, only one infusion was included in the study. IVIG was given to patients for primary immunodeficiencies (n = 8), ITP (n = 65), Kawasaki disease (n = 11), secondary immunosuppression (n = 28), and passive immunization (n = 3).

Headache, fever, chills, nausea, rash, arthralgia, myalgia and back pain were accepted as mild immediate events; wheezing, chest pain as moderate and anaphylaxis, hypotension, cardiovascular events, altered mental status as severe reactions.

Demographic data (age, gender), co-morbid diseases, history of reactions to blood products / IVIG were taken from the parents and their primary clinicians.

IVIG preparations at concentrations with 5% and 10% and pentaglobin were used. Infusion rates were between 3-10 ml/kg/hour. When mild reactions were seen, infusion was paused temporarily and re-started after complete resolution of the symptoms. In the course of moderate and severe reactions, infusion was stopped totally.

Findings were analysed using chi-square test with SPSS 11.0 statistics programme, and difference was considered significant below the p = 0.005 level.

The local Ethics Committee of Bakirkoy Research and Training Hospital has approved the study.

Results

One hundred and fifteen patients (n = 115) were included into the study. There were 62 (54%) boys and 53 (46%) girls aged between 1 month - 18 years. Mean age of the group was 7.4 ± 4.6 years (median: 6 years).

Immediate adverse events due to IVIG infusions were seen in 29 (25.2%) of the patients. Some of them appeared during infusion, and some was reported by parents at the next visit. Demographic features, diseases requiring IVIG treatment and the rate of immediate adverse events were summarized in **table 1**. Gender and types of the diseases were not different in significance regarding the presence of adverse events (p = 0.278 and p = 0.936, respectively) (**table 1**). Strikingly, in primary immunodeficiency patients, percentage of adverse events was higher than the other groups in percentages (50% of the 8 patients). There was no significant association between the rate of adverse reaction and age in patients with primary immunodeficiency disorders.

Thirty-eight (33%) patients had received IVIG or other blood product infusions previously, and only 6 of them had mild reactions in previous infusions. Pre-medication with anti-histamines and anti-pyretics were performed in those 6 patients who described previous adverse reactions. In 3 of those 6 patients, adverse events recurred. Although the number of the patients was low, the rate of adverse events did not change with pre-medication (p = 0.16). In addition, previous history of receiving blood product and having reaction to IVIG or another blood product did not show significant difference in developing adverse events (p = 0.52, p = 0.68, respectively).

Ninety-five percent of the patients were treated with 5% IVIG preparations, so we could not perform statistical analysis between the other concentrations of IVIGs. **Table 2** shows the types of immediate adverse reactions to different IVIG preparations. There was no difference between various IVIG solutions regarding the types of immediate adverse reactions.

Table 1 *- Demographic features of patients and types of diseases.*

	Patient number (n, %)	Adverse reaction (n, %)	p
Gender			
Female	53 (46)	15 (28.3)	0.278
Male	62 (54)	14 (22.6)	
Replacement (0.4-0.5 gr/kg)			
Primary Immunodeficiencies	8 (7)	5 (62.5)	0.936
Secondary Immunosuppression[1]	28 (24)	3 (10.7)	
Immunomodulation (1-2 gr/kg)			0.936
Kawasaki Disease	11 (9.6)	2 (18.2)	
Autoimmune Diseases[2]	65 (56)	19 (29.2)	
Other[3]	3 (2.6)	-	

[1]Hemophagocytic hystiocytosis (HLH), Malignancy.
[2]Guillain-Barré Syndrome, Immune thrombocytopenic purpura (ITP).
[3]Passive immunization for measles exposure.

Table 2 *- Immediate adverse reactions and association with different IVIG preparations.*

n (%)	Octagam	Flebogamma	IgVena	Pentaglobuline	Tegeline	Kiovig	Total of all adverse events
Fever / Chills	4 (30.7)	8 (61.5)	1 (7.6)	-	-	-	13 (44.8)
Vomiting	4 (40)	3 (30)	2 (20)	-	-	1 (10)	10 (34.5)
Headache	2 (28.5)	2 (28.5)	2 (28.5)	-	-	1 (14.2)	7 (24.1)
Nausea	1 (3.5)	1 (3.5)	-	-	-	-	2 (6.9)
Rash	-	-	-	-	1 (3.5)	1 (3.5)	2 (6.9)
Wheezing	-	-	-	-	-	1 (3.5)	1 (3.5)
Ventricular fibrillation	-	-	-	1 (3.5)	-	-	1 (3.5)

Table 3 *- Adverse reactions according to types of diseases.*

n (%)	Kawasaki Disease	Autoimmune Diseases	Primary Immunodeficiencies	Secondary Immunodeficiencies	Total of all adverse events
Fever / Chills	2 (15.3)	8 (61.5)	2 (15.3)	1 (7.6)	13 (44.8)
Vomiting	-	9 (90)	-	1 (10)	10 (34.5)
Headache	-	7 (100)	-	-	7 (24.1)
Nausea	-	1 (50)	1 (50)	-	2 (6.9)
Rash	1 (50)	-	-	1 (50)	2 (6.9)
Wheezing	-	1 (100)	-	-	1 (3.5)
Ventricular fibrillation	-	-	-	1 (100)	1 (3.5)

The most common immediate adverse reactions were fever and chills (13% of the total adverse events). In 6 patients, more than one adverse reaction were observed such as fever and headache. During the first 5 minutes of pentaglobuline infusion, ventricular fibrillation and cardiac arrest developed in 1 patient, but this patient was in septic shock status and clinically unstable. When we look at the adverse events one by one, none of them differed according to types of the diseases ($p > 0.05$). But we saw that headache was only seen in patients receiving IVIG due to ITP (**table 3**). Moderate events were rare, only 1 patient developed wheezing. Mild reactions such as headache continued over 6 hours in 2 patients. Except for these one moderate and one severe reaction, all remaining adverse events were accepted as mild. Immediate adverse reactions were seen in the first 6 hours of infusion in 7 patients and during infusion in the remaining 22 patients. They were treated with slowing of the infusion rate and infusion was stopped in 3 patients because of moderate events in 1 patient and not responding to slow the rate of infusion in 2 patients. Medical treatment with anti-histamine and/or anti-pyretic was required in 14 patients (48%) after adverse events occurred.

Discussion

The severity of the adverse events symptoms can be examined as mild, moderate and severe (8). We accepted mild reactions as headache, fever, nausea, emesis, flushing, muscle ache, chills, feeling sick, itching, urticaria, anxiety, light headedness, moderate reactions as chest pain, wheezing and worsening symptoms of mild reaction and severe reactions as persisting or worsening of moderate reactions and severe headache and shaking, severe breathlessness or wheezing, severe dizziness or fainting, sensation of pressure in chest or collapse. We evaluated only immediate adverse events in this study. Most of the patients developed only mild reactions. Severity of the adverse events were not found significantly different from the literature (13).

Mild reactions were subsided with decreasing the infusion rate, and it can also be managed by medications such as antihistamines, paracetamol and small doses of corticosteroids, but in moderate reactions it is necessary to stop the infusion. Adrenaline administration and further medical attention would be required in severe reactions. It was reported that adverse reac-

tions were frequently seen in non-primary immune deficiency patients (13). In our patients, although there was no difference between adverse events and types of diseases, the percentage of adverse events in primary immunodeficiencies were seen higher. Adverse reactions with IVIG infusions vary in wide range, but mostly about a rate of 20% (3,6). In our study, adverse events were seen at a rate of 25.2%, this finding was similar with the literature. Higher infusion rates are reported to be related to higher adverse events. Beginning the infusion slowly, and then increasing gradually, based on patient's tolerance, can prevent these reactions (14). In our study, as recommended, we started at minimal infusion rates and raised to 10 ml/kg/h to minimize the adverse effects. Unfortunately, because of the lack of previous data, we could not compare the infusion rate and adverse event rates.

In the literature, one of the most common adverse event was reported as persistent headache due to acute aseptic meningitis. Headache can be minimized by continuing IVIG therapy at a slow infusion rate and by hydration, antihistamines and analgesics (15). It is also suggested that there was a relationship between high and concentrated dose of IVIG and headache (15,16). We did not show a correlation between higher dose and more frequent headache. Headache was not the most common adverse effect in our study and this can be related to the less usage of the high concentrated IVIG preparations. The other most common adverse reaction is reported as fever (17). Fever was the most common adverse reactions in our study and this was compatible with the literature.

Bichuetti-Silva *et al.* also noticed in their study that some patients had more than one adverse reaction at the same infusion (13). Similar with this study, 6 patients (5%) had more than one adverse events in our study. Hamrock et al. reported that premedications before IVIG infusions may prevent the adverse reactions (18). In our study, 38 patients had received IVIG or blood product infusion previously, six of them have had adverse events more than one time despite premedication with diphenhydramine and acetaminophen before IVIG infusions, in half of them adverse events recurred. Premedication may reduce the incidence of adverse events and we suggest to use premedication especially in patients who are receiving IVIG regularly.

Existing infection during the infusions was reported as an important risk factor for IVIG adverse effects. The reason of this was explained the antigen-antibody complex formation during the infection. Adverse reactions occur probably due to aggregated immunoglobulin molecules which cause the complement system to be activated, antigen-antibody reactions, possible contaminants or even stabilizers that may have been used during the manufacturing process (17). In our study, IVIG was used for secondary immunosuppression in 28 patients and passive immunization for measles in 3 patients. Unlikely, despite severe infections in secondary immunosuppressive patients, existing infection did not increase the rate of adverse events.

In summary, because of the increasingly use of IVIG therapy, it is important to know the adverse events. It should be kept in mind that adverse events to IVIG might be more frequent and severe with high dose, high infusion rates, during infection period and especially in primary immunodeficiency diseases. In addition, records related to IVIG infusions should be noted properly to set the infusion rates and doses in the next infusions.

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Hymenoptera Venom Allergy. A closer collaboration is needed between allergists and emergency physicians

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Key words

hymenoptera venom allergy; anaphylaxis; specific immunotherapy; emergency; prevention

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Summary

Background. *Hymenoptera stings are sometimes fatal in venom-allergic patients. Fatalities mostly occur in previously stung subjects, especially those with a history of systemic reactions, and could be avoided if patients were properly informed of the existence of a prevention strategy for insect stings, referred to an allergy follow-up and prescribed auto-injectable epinephrine and/or venom-specific immunotherapy (VIT). We sought to assess knowledge and awareness of Hymenoptera Venom Allergy (HVA) in a small sample of Emergency Physicians in our geographic area.* ***Methods.*** *An eight-point questionnaire on HVA was administered to Emergency Department physicians working in the six largest ED in Naples.* ***Results.*** *Twenty-seven physicians completed the questionnaire. Twenty/27 (74%) were unaware of the classification of Hymenoptera sting reactions, 11/27 (41%) were unaware of the existence of prevention strategies such as VIT, 18/27 (67%) did not refer HVA patients to a specialist follow up. One/27 (4%) prescribed auto-injectable epinephrine and 100% wish better information on the topic.* ***Conclusions.*** *In our survey we found a number of ED physicians whose knowledge of HVA, beyond the emergency treatment, is not satisfactory. A closer collaboration among ED physicians and allergists is urgently needed.*

Introduction

Allergic systemic reactions to Hymenoptera stings cause significant morbidity, impairment of quality of life, and are sometimes fatal. Many fatalities occur in previously stung subjects, especially in those with a history of systemic reactions (1). Hymenoptera Venom Allergy (HVA) patients who have experienced an anaphylactic reaction and also have detectable venom - specific IgE or positive skin test - should be prescribed a Venom Specific Immunotherapy (VIT) (2). An allergy follow-up after an episode of anaphylaxis is advisable in preventing further episodes (3-5), and supports current anaphylaxis guidelines (6). Nevertheless, the number of patients with anaphylaxis who are correctly followed-up after the treatment in Emergency Department (ED) remains low, and a closer collaboration between ED physicians and allergists has become an urgent need (5). In our outpatient activity we noticed that most HVA patients come spontaneously, after having been stung two or more times, while few patients are referred to our Allergy Unit after treatment in an ED. Our survey aims at assessing the level of knowledge and awareness of HVA among ED Physicians of Naples and surroundings area.

Methods

We organized a one-afternoon meeting on anaphylaxis and sent an invitation to the six largest hospital EDs in Naples metropolitan area. All the ED physicians who were present at the meeting were invited to anonymously complete an 8-point questionnaire concerning different aspects of HVA that we had previously prepared (see **table 1**). All the questionnaires were filled and collected before attending the meeting. In order to reduce the risk of selection bias all the doctors who were not on duty in that day were asked to come to the meeting.

Results

Twenty-seven physicians participated in the study. All of them were specialized in Emergency Medicine. All of them completed the questionnaire. As result, very few of the ED physicians who participated in the study demonstrated a good knowledge of HVA. Twenty/27 (74%) were unaware of the classification of Hymenoptera sting reactions (7). Eighteen/27 (67%) do not refer HAV patients to a specialist follow up. Twenty-five/27 (93%) give epinephrine as first-line treatment of anaphylaxis but only 1/27 (4%) had ever prescribed auto-injectable epinephrine. Finally, 100% of physicians wish better information on the topic (**table 1**).

Discussion

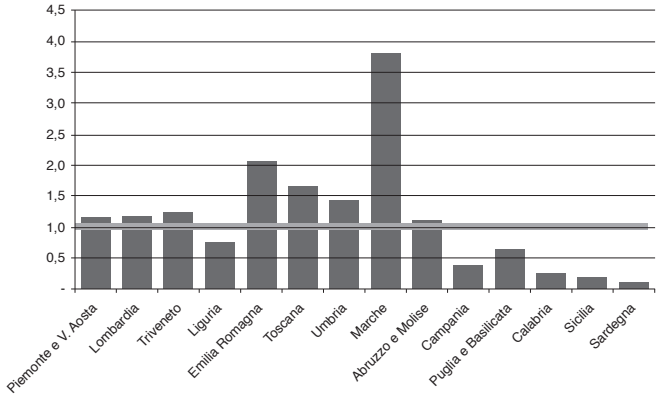
This is the first time that such a survey has been performed in Italy. Our experience suggests that among ED physicians, at least in Naples area, there may be insufficient knowledge of the risks associated with HVA. Eighteen doctors (67%) admit they don't refer the patient to the specialist follow-up, which is consistent with the results of other studies (6), that means two-thirds of patients receive little or no information about a preventive strategy. In contrast with some studies (8), twenty-five out of the twenty-seven ED doctors (93%) administer epinephrine as first-line treatment of anaphylaxis. Only one in the study group had ever prescribed auto-injectable epinephrine. These data are generally consistent with the low rate of auto-injector prescriptions worldwide (9), specifically in EDs (10) and the fact that in Italy ED Physicians cannot prescribe auto-injectable epinephrine free of charge. In Italy only Allergists can do so. In Italy there are some regions such as Marche or Toscana in which the number of VIT prescription is several fold higher compared to other regions such as Campania or Calabria and southern Italian regions in general (www.assobiomedica.it). At least two explanations seem plausible in accounting for this discrepancy. Both are disheartening. First, many patients from southern Italy are forced to travel north in order to get their prescription, which carries both a financial and social burden. The second and even more disturbing is that most patients are simply lost at follow-up. VIT prescription is mandatory for those patients who have a history of anaphylactic reaction after a hymenoptera sting and who also have detectable venom-specific IgE (6). Therefore, we believe that the number of VIT/year/million inhabitants (a sort of "VIT Index") may be considered an indirect marker of the clinicians' awareness level in a particular region (**figure 1**).

Our study has some limitations. Firstly, the small number of interviewed physicians who made up a group was geographically limited to the confines of the Neapolitan area. For this reason, we are now carrying out a larger randomized study involving ED Physicians and General Practitioners from other Italian regions. Another point of weakness is the study population selection. We knew that a meeting on anaphylaxis might result in attracting a specific group of physicians, either because they

1	Do you know how to classify the Hymenoptera sting reactions?	Y	7 (26)
		N	20 (74)
2	Do you prescribe auto-injected epinephrine?	Y	1 (4)
		N	26 (96)
3	Do you know how likely it is an anaphylactic shock in a patient with a former Systemic Reaction?	Y	9 (33)
		N	18 (67)
4	Do you know if a long term prevention strategy for HVA patients does exists?	Y	16 (59)
		N	6 (22)
		?	5 (19)
5	Do you usually send the HAV patients to an Allergy Unit?	Y	9 (33)
		N	18 (67)
6	Do you utilize Epynephrine as first line treatment for anaphylaxis?	Y	25 (93)
		N	2 (7)
7	Which is, to your opinion, the best way for the epinephrine injection?	s.c.	2 (7)
		i.m.	13 (48)
		e.v.	12 (45)
8	Would you like to be better informed about the problem?	Y	27 (100)
		N	0 (0)

are already quite knowledgeable about the issue or because they recognize gaps in their knowledge on the topic. We sent the meeting invitation to the six larger ED departments of our area. Only those physicians who were free of work commitments that day could participate. This should have reduced the risk of selection bias. We are aware that twenty-seven doctors cannot be considered representative of all ED physicians in the Naples area and that many other emergency physicians in our area might have had a better knowledge of the subject. Our only scope is to bring to the general attention the fact that the problem may, in some areas, be real. One could say that this is not a worldwide problem and that our study just reflects a small, geographically limited problem. Of course this may be true but nevertheless we believe that allergists and ED physicians should reflect and work together in order to verify the level of awareness of HVA in their own working area.

Figure 1 *- VIT Index (% VIT prescription / % inhabitants). Gray line (VIT index = 1,0) represents the equilibrium between the percentage of inhabitants and the number of VIT prescriptions for each Italian region. Values > 1: regions where the prescriptions are more than expected. Values < 1: Regions where the prescriptions are lower than expected. For example, Piemonte has 8% of Italian population and 8.1% of prescription. Campania has 10.2% of Italian population and 3.3% prescription, Marche has 2.6% of Italian population and 10% VIT prescription. Data refer to the whole Italian market (courtesy Anallergo, Florence, Italy).*



Conclusion

Many cases of Hymenoptera venom-induced anaphylaxis following a first-time reaction can be safely avoided through the correct preventive strategy. After ED physician treat the emergency event, patients should be referred to an Allergy Unit in order to assess their eligibility for VIT and for prescription of auto-injectable epinephrine. Our experience suggests that among ED physicians, at least in some areas, there may be insufficient knowledge of the risks associated with HVA and that better information is needed. Allergists should notice if the number of HVA patients they treat in their Allergy Unit or the VIT prescriptions number in their working area are some lower than expected. A closer collaboration between allergists and ED physicians may help patients with HVA who had experienced an anaphylaxis to avoid further life-threatening episodes in the future.

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Ara h 6 sensitization in peanut allergy: friend, foe or innocent bystander?

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Key words
Ara h 6; Ara h 2; Ara h 9; component resolved diagnostic; peanut allergy

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Summary
The clinical role of Ara h 6 sensitization in peanut allergy is a current matter of debate. We investigated the role of Ara h 6 sensitization patterns in a sample of young adults from different Italian cities. Sera of 33 patients with specific IgE against Ara h 6 were selected. According to clinical symptoms upon peanut ingestion, patients were divided into severe reaction (SR) and mild-tolerant (MT) subgroups. While the SR group mainly showed sensitization patterns involving Ara h 2 and other major allergenic components, a previously undescribed association between Ara h 6 and Ara h 9 was found in the MT group. This pattern seems to be clustered in Mediterranean Italy and associated with Pru p 3 sensitization. This finding might shed a new light on the role of Ara h 6 sensitization in peanut allergy.

Introduction

Food allergy is a growing epidemic in Western countries, affecting mostly children and young adults, with a severe impairment of the quality of life and a potentially fatal outcome.
In clinical practice there is a strong need for prognostic markers to better identify subsets of patients at high risk of anaphylaxis, allowing earlier recognition and proper treatment. Current research is trying to unveil the association between the severity of food allergy and the detection of IgE antibodies against specific allergenic components. Hence component-resolved diagnostic (CRD) is playing a greater role in the diagnostic workup of food allergy.
Concerning peanut allergy (PA), several studies showed how the sensitization to various seed protein families has a diverse impact on the clinical outcome upon allergen exposure. The sensitization to 2S albumins, like Ara h 2, is predominant in peanut allergic children from USA and continental Europe, and is highly linked to severe allergic reactions compared to PR-10 (Ara h 8) and Lipid Transfer Protein (LTP) (Ara h 9) sensitizations (1). In the Mediterranean area the sensitization to Ara h 9, a non-specific LTP allergenic molecule, is the most frequently observed cause of PA (1-4). Ara h 9 sensitization was seen to occur mostly in areas where the sensitization to other LTP molecules, in particular Pru p 3, were also observed (1). Furthermore, a cross-inhibition study performed on sera of patients with peanut and peach allergy suggested that Pru p 3 sensitization acts as a primary sensitizer for Ara h 9, confirming the strong correlation between these two molecules (3).
Ara h 6 is a seed storage protein belonging to the 2S albumin family that shares structural homology with Ara h 2. Ara h 2 and Ara h 6 sensitizations occur often simultaneously and both share the same clinical features and prognostic value in peanut allergic subjects (5-7). The detection of both allergenic molecules is considered by some to be redundant, especially in adults

(1,5). However, the combined Ara h 6 - Ara h 2 determination yielded a better diagnostic performance than single sensitizations in high-risk children (7,8) and there is small evidence that anaphylaxis can occur even in Ara h 6 mono-sensitization (9). Although there are still some gray areas in the use of microarray technology in CRD (10,11), multiplex assay offers exciting opportunities for broad range IgE testing and identification of sensitization patterns in PA. Moreover, a commercial microarray panel including the Ara h 6 molecule is currently available for diagnostic purposes in clinical practice. The aim of this study was to assess the sensitization patterns involving Ara h 6 and their clinical role in a sample of children and young adults from different Italian cities.

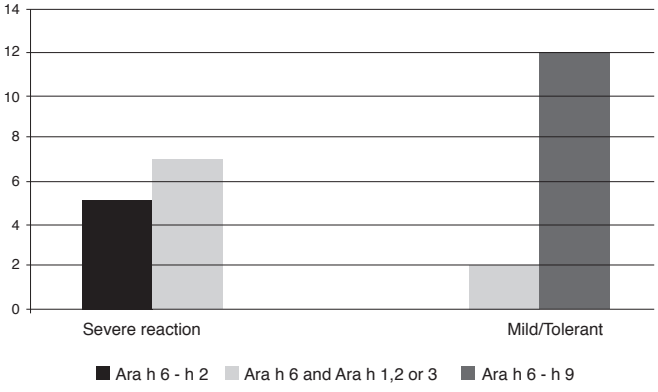
Materials and Methods

We retrospectively analyzed microarray test results of sera collected from three Italian allergy centers of different climatic regions: northern/continental area (Pordenone) and central/southern Mediterranean areas (Ancona and Naples).
Specific IgE against Ara h 6 were assessed using ImmunoCAP® Immuno Solid-phase Allergen Chip 112 (Thermo Fisher, Uppsala, Sweden).
We selected patients with specific IgE against Ara h 6 and absence of sensitization to cross-reactive carbohydrate determinants (MUXF3) and we collected data on clinical history, type and severity of allergic reaction upon peanut exposure and skin prick test (SPT) results for peanut extract.
Patients were then divided according to clinical history into two groups: the severe reaction group (SR) included any patient who reported at least two of the following symptoms upon peanut exposure: hypotension, syncope, urticaria, dyspnea, vomiting.
In the mild reaction or tolerant group (MT) were included all subjects with mild local symptoms (i.e. oral allergy syndrome) or no symptoms at all after peanut ingestion.
A commercial peanut extract (ALK-Abelló, Madrid, Spain) was used for SPT, along with positive (histamine 10 mg/mL) and negative (saline solution) controls. A positive SPT was defined as ≥ 3 mm wheal diameter compared to negative control.
Patients whose SPT results or clinical history were unavailable, or tested negative for peanut extract were excluded from the study. Positivity threshold were set to ≥ 0.30 ISU for Ara h 6 and ≥ 0.00 ISU for MUXF3.
Statistical analysis was performed using Microsoft® Excel 2007 (Microsoft, Redmond, USA).

Results

Sera of 74 patients were analyzed, though only 33 subjects fulfilled the enrollment criteria (mean age 16.5 ± 9.4 years; 11 females). Among these, 16 subjects experienced severe reaction to peanut, 3 mild local symptoms and 14 tolerated peanut consumption. Five of 16 SR patients showed sensitization to Ara h 6 and Ara h 2, while in the MT group this association was not seen. By contrast, Ara h 6 and Ara h 9 co-sensitization was present in 12 out of 17 MT patients and none of the SR group (*P* value = 0.002, Fisher exact test).
The statistical difference between SR and MT groups was significant even when Ara h 6 and the co-sensitization to other major peanut allergens associated with severe PA (Ara h 1, Ara h 2, Ara h 3) was considered (**figure 1**).
Mean Ara h 6 IgE levels in the SR group were significantly higher compared to the MT group (8.3 ± 9.1 vs 2.8 ± 3.3 ISU, *P* value < 0.005) (**table 1**). Conversely, the Ara h 9/Ara h 6 IgE ratio was considerably higher in MT patients compared to SR (1.8 vs 0.1). Each patient co-sensitized to Ara h 6 and Ara h 9 showed specific IgE against Pru p 3 and the Ara h 6 - h 9 pattern was present in the Ancona and Naples centers only. On the contrary, the Ara h 6 - Ara h 2 co-sensitization pattern was scattered all over Italy (**figure 2**) and only three patients of the SR group from Naples showing the Ara h 6 - h 2 pattern were also sensitized to Ara h 9/Pru p 3.
No difference in age (median age SR vs. MT: 12 vs 15 years; p > 0.05, Student T-test) or sex was seen between Ara h 6 - h 9 and Ara h 6 - h 2 co-sensitized groups.

Figure 1 *- Distribution of patients according to the sensitization pattern and clinical severity of peanut allergy.*



	*p**
Ara h 6 - 1,2 or 3 vs Ara h 6 - 2	NS
Ara h 6 - 1,2 or 3 vs Ara h 6 - 9	0,0003
Ara h 6 - 2 vs Ara h 6 - 9	0,0002

* Fisher exact test, SR vs MT
Abbreviations: MT, mild/tolerant group; NS, not statistically significant; SR, severe reaction group.

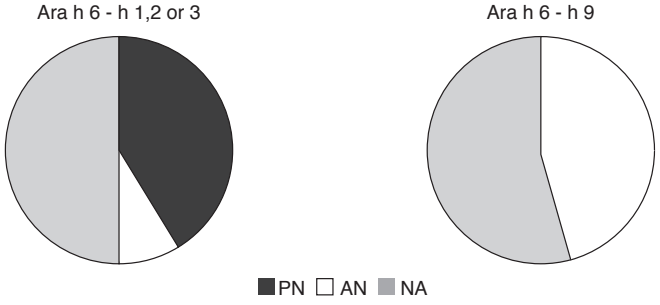
*Fisher exact test, SR vs MT
Abbreviations: MT, mild/tolerant group; NS, not statistically significant; SR, severe reaction group.

Table 1 - *Specific IgE levels for Ara h 2, Ara h 6, Ara h 9 and Pru p 3 seen in the severe reaction and mild / tolerant groups.*

		SR	MT	P value
Ara h 2	mean	11.1	0.9	< 0.05
(ISU)	SD	15.3	3.1	
Ara h 6	mean	8.3	2.8	< 0.05
(ISU)	SD	9.1	3.3	
Ara h 9	mean	1.1	5.2	< 0.05
(ISU)	SD	3.7	5.2	
Pru p 3	mean	1.1	9.4	< 0.005
(ISU)	SD	2.6	11.0	

[1]Student T test, SR vs MT.
Abbreviations: ISU, ISAC Standardized units; MT, mild/tolerant group; SR, severe reaction group.

Figure 2 - *Distribution of main Ara h 6 co-sensitization patterns observed according to center.*



Abbreviations: PN, Pordenone; AN, Ancona; NA, Naples.

Discussion

Our study confirms the known association between severe allergic reaction to peanut and the Ara h 6 - h 2 sensitization pattern. Furthermore, it suggests the presence of a new pattern, namely Ara h 6 - h 9, associated with mild or none clinical symptoms upon peanut exposure, clustered in Mediterranean Italy.

These results therefore question the close relationship between Ara h 6 sensitization and severe reaction to peanut. Two aspects of this finding are noteworthy: the geographical clustering of the sensitization patterns and the impact of the co-sensitization profile on the prognostic outcome of Ara h 6. Italy is largely considered a Mediterranean country, however there are marked differences between northern and southern regions in terms of climate. These regional differences are mirrored by the diverse clinical manifestations and sensitization patterns seen in both respiratory and food allergy (12,13).

Peanut allergy apparently makes no exception; we observed a strong resemblance between the sensitization patterns seen in Pordenone and continental Europe in peanut allergic subjects (1). While the Ara h 6 - h 2 pattern was evenly distributed throughout the country, we speculate that the observed predominance of this pattern in continental areas is due to the lack of sensitization to Pru p 3, as seen in Northern Italy. On the other hand, the distribution of Pru p 3 sensitization clearly overlapped Ara h 6 - h 9 sensitization in Southern Italy, but surprisingly the Pru p 3/Ara h 9 co-sensitization was seen to seldom occur in Ara h 6 - h 2 positive patients from southern regions. Therefore, our data support that sensitization patterns, rather than single sensitizations, are better means to assess the prognostic value of IgE positivity in food allergy, a concept that was already described in peach allergy (14,15). Several hypothesis can be drawn to better explain the prognostic shift of Ara h 6 according to the concomitant sensitization pattern. We observed an inversion of the Ara h 9/Ara h 6 ratio in the SR vs MT group, and this might suggest that the presence of a high level of specific IgEs against LTP molecules might have a role on the sensitization to Ara h 6. Although it has never been demonstrated to date, some degree of cross-reactivity between Ara h 6 and Ara h 9 could ensue, being both proteins belonging to the prolamine superfamily (16). Furthermore, we noted a marked difference in Ara h 6-specific IgE levels between the Ara h 6 - h 2 and Ara h 6 - h 9 patterns, and this may be an additional reason for the different clinical outcomes observed. We applied a positive cut-off value for Ara h 6 of ≥ 0.30 ISU as suggested by the manufacturer, although other authors showed better sensitivity and specificity of microarray Ara h 6 IgE assay when applying a higher threshold (≥ 2.00 ISU) (8). Eventually, we cannot exclude the possibility of a false positive result linked to the detection system in the presence of high level LTP sensitization.

Since the Ara h 6 - Ara h 9 pattern was not reported in recent studies on Ara h 6 sensitization in both pediatric and adult patients from Mediterranean areas (1,9), the reasons for this discrepancy are worthy of discussion. The different age groups considered might explain the inconsistency between our results and those shown by Pedrosa et al (9), where Ara h 9 sensitization was present only in a small percentage of children with no correlation with Ara h 6. Our sample belongs to an older age group compared to Pedrosa's pediatric population (mean age 16.5 ± 9.4 years vs 7.62 ± 3.23 years) (9). Although conflicting results on Pru p 3 allergy in children were documented (17,18), the onset of Pru p 3 sensitization seems to occur predominantly after early childhood (4). A second discrepancy was found between our study and the results of the EuroPrevall cohort, designed to describe the sensitization patterns in PA across Eu-

rope (1). In this survey 18 patients (12% of the overall sample) were enrolled in Mediterranean areas (Spain, Italy and Greece) (1). Though our cohort shares with this selected subgroup the same age and geography, the Ara h 6 - Ara h 9 sensitization pattern was not reported. We speculate two possible reasons for divergence, namely a selection bias or a difference in Ara h 6 IgE detection techniques. Concerning the first, in the EuroPrevall survey patients were selected by oral peanut challenge and the number of Mediterranean patients, both tolerant and non-tolerant, was small and this could have led to an underestimation of the Ara h 6 - h 9 pattern. On the contrary, in our study the selection was driven by the detection of specific IgE against Ara h 6, which could have magnified the Ara h 6 - h 9 subset.
As for IgE detection techniques, for the present study we performed a multiplex assay available for diagnostic purpose in clinical setting, while the EuroPrevall group developed an experimental singleplex assay for Ara h 6 IgE testing and concerns on the reliability of EuroPrevall's experimental assay have already been expressed (9).

Conclusion

Multiplex CRD performed in Italian Ara h 6-positive patients showed two main sensitization patterns, namely Ara h 6 - h 2 and Ara h 6 - h 9, with different clinical outcomes. Our study offered new insights on PA and Ara h 6 sensitization in Mediterranean areas, emphasizing the role of sensitization patterns, rather than single IgE positivity, for prognostic purposes. Limitations of this study were the retrospective design, the small number of observations and the absence of an oral peanut challenge in self-reported tolerant subjects. Further studies are needed to properly elucidate the impact of LTP allergy on Ara h 6 sensitization.

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High-dose nebulized budesonide is effective for mild asthma exacerbations in children under 3 years of age

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Key words
Asthma; budesonide; exacerbation; infants; steroid

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Summary
Background. *High-dose inhaled steroid therapy has been shown to be effective in children and adults with asthma exacerbations. However, few reports are available regarding its efficacy for asthma exacerbations in younger children.* ***Objective.*** *In this study, we administered high-dose nebulized budesonide therapy for mild asthma exacerbations in children < 3 years of age and compared its efficacy and safety with systemic steroid therapy.* ***Methods.*** *This study included children < 3 years old with mild asthma exacerbations. Patients were randomly assigned to two groups: the BIS group was given 1 mg of nebulized budesonide twice daily, and the PSL group received prednisolone 0.5 mg/kg iv three times daily. Days to disappearance of wheezing, days of steroid use, days of oxygen use, serum cortisol level, and incidence of adverse events during treatment were compared between the groups.* ***Result.*** *Wheezing disappeared after an average of five days, and steroids were administered for an average of five days in both groups, with no significant difference in days of oxygen use. Serum cortisol levels at initiation and during the course of treatment remained unchanged in the BIS group, and were decreased in the PSL group; however, the decrease in the latter group was not pathologic.* ***Conclusion.*** *For children < 3 years old with mild asthma exacerbations, high-dose nebulized budesonide therapy is equally as effective as systemic steroid therapy.*

Introduction

Since 1990, when nebulized budesonide was first approved, inhaled steroid therapy for asthma in infants has been widely used. Inhaled steroid therapy is considered the best treatment for long-term management of asthma, and has been reported to stabilize clinical symptoms, improve airway obstruction and quality of life, and reduce the frequency and progression of asthma exacerbations (1). Even for asthma exacerbations, high-dose inhaled steroid therapy has been reported to be as effective as systemic steroid therapy, mainly in school-age and older patients (2).

The main reason why high-dose inhaled steroid therapy is not commonly used for the treatment of acute asthma exacerbations in children below school age, is that repeated episodes of wheezing in younger children are often due to viral infections and that there are no clear differences of clinical symptoms between asthma exacerbation and acute bronchiolitis. However, the majority of children with asthma experience their first episode of wheezing as an infant, and these initial illnesses are almost always caused by viral infections (3). Early diagnosis and early intervention for asthma in infants are mandatory to maintain their airway function.

In this study, we carefully recruited infants with asthma according to the criteria of the Japanese Society of Allergology, which predicts persistent asthma in children less than 3 years of age and administered high-dose nebulized steroid therapy to children less than 3 years of age with exacerbations of asthma. The

efficacy and safety of inhaled steroid therapy is compared with conventional systemic steroid therapy.

Methods

Patients

This study enrolled children less than 3 years of age, diagnosed with bronchial asthma as defined by the Japanese Society of Allergology as follows: children with at least three repeated episodes of obvious expiratory wheezing and children satisfying any of the following six criteria with recurrent wheezing more than 2 episodes: 1. At least one parent of the child was diagnosed with bronchial asthma by a physician, or tested positive for IgE antibodies specific to an inhaled antigen; 2. The child was diagnosed with atopic dermatitis by a physician, or tested positive for IgE antibodies specific to an inhaled antigen; 3. The child or any member of his/her family has high serum level of IgE; 4. Eosinophils or creola bodies are found in the sputum; 5. The child has had an episode of wheezing in the absence of an apparent airway infection or 6. An improvement in wheezing or labored respiration, or an improvement in oxygen saturation was observed after inhaled beta 2-agonist. These children first received intravenous hydrocortisone and a single inhalation of procaterol for an asthma exacerbation. If they showed inadequate improvement in clinical symptoms, they were admitted with the diagnosis of a mild asthma exacerbation.

Study protocol

Children were randomly divided by a computer into a high-dose inhaled steroid therapy group (BIS group; nebulized budesonide 1 mg/dose, twice daily inhalations) and a systemic steroid therapy group (PSL group; intravenous prednisolone 0.5 mg/kg, three times daily) at the time of admission, and treated accordingly. Inhalation was conducted using PARI turbo BOY N (PARI international, Starnberg, Germany) with a face mask, and concluded when the aerosol was no longer visible. The amount of inhaled or injected steroid was gradually reduced from the day after disappearance of wheezing, and then discontinued. Children confirmed to have no recurrence of expiratory wheezing were discharged. During hospitalization, children continued to receive long-term asthma medication other than steroids and procaterol inhalation four times daily. Long-term asthma medication was administrated according to the guideline proposed by the Japanese Society of Allergology as follows: Children who have wheezing episodes less than once a month receive intermitted LTRA; Children who have wheezing episodes at least once a month but less than once a week receive daily LTRA; Children who have wheezing episode at least once a month with above treatment are added inhaled steroid. As post-discharge treatment, children who had asthma exacerbation within a month before their hospitalization received additional inhaled steroid or increased dosage of inhaled steroid along with LTRA. When percutaneous oxygen saturation was less than 94%, oxygen inhalation was added according to guideline by the Japanese Society of Allergology. Antibiotics were used when a bacterial infection of the respiratory tract was suspected. Children requiring mechanical ventilatory management, children with chronic systemic diseases or primary lung diseases, and children whose guardian did not consent to study participation were excluded. Clinical symptoms, the presence or absence of wheezing, percutaneous oxygen saturation, treatment details, and routine clinical data were recorded daily, and serum cortisol levels were measured at the time of admission, and between 8 am and 10 am at four days after admission.

Data collection

The primary outcome of this study was the number of days to disappearance of wheezing, and secondary outcomes included the number of days of steroid use, the number of days of oxygen use, presence of suppressed serum cortisol levels during the hospital course, and the incidence of adverse events.

Statistical analysis

Statistical analysis was carried out with JMP 9.0.0 software (SAS Institute, Cary, NC, USA). Student's t-test was used to compare values between the two groups, and Fisher's exact test was used to compare proportions between the two groups. A difference was considered significant with $p < 0.05$. Welch's test was used for total serum IgE and the peripheral blood eosinophil ratio.
This study was conducted with approval from the ethical review committee of this institution, and written consent was obtained from a guardian of each child after being provided with an explanation about the study by the physician who determined that the patient required inpatient treatment.

Results

Clinical characteristics

Fifty-one children with a mild asthma exacerbation admitted to the Department of Pediatrics at the Haga Red Cross Hospital (Tochigi, Japan), between April 2013 and November 2014 met the inclusion criteria, but one refused to participate. Thirty patients in the BIS group and 20 in the PSL group completed all follow-up including post-discharge follow-up. The mean ages were 20 and 21 months in the two groups, and the number of

Table 1 *- Patient background.*

	BIS (n = 30)	**PLS (n = 20)**	
Age (months)	20 ± 2	21 ± 2	
Males:Females	21:9	15:5	
Weight (kg)	11 ± 0	12 ± 0	
Height (cm)	81 ± 1	83 ± 2	
BA controller			
LTRA	18	15	
LTRA+ICS	11	4	
ICS	1	0	
Intermitted LTRA	11	9	
Total serum IgE (IU/mL)	160 ± 90	330 ± 100	
Respiratory rate at admission (/ min)	34 ± 1	34 ± 1	
SpO_2 on admission (%)	97 ± 0	96 ± 1	
Febrile patients	15	7	
Antibiotics therapy	4	2	
Laboratory findings			
WBC (/μL)	12100 ± 720	10700 ± 890	
Eosinophil (%)	2.9 ± 0	1.7 ± 0*	*$p = 0.0183$
Platelets (x10^4 /μL)	31 ± 2	27 ± 2	

BIS: high-dose inhaled budesonide therapy group; PSL: systemic steroid therapy group; BA: bronchial asthma; LTRA: leukotriene receptor antagonist; ICS: inhaled steroid; SpO_2: percutaneous oxygen saturation; WBC: white blood cell count.

male patients was greater than the number of females in both groups (**table 1**). The peripheral blood eosinophil ratio was significantly higher in the BIS group (BIS group 3.0%, PSL group 1.7%; p = 0.0183). No significant differences were noted in other clinical data, including total serum IgE, respiratory rate on admission, and percutaneous oxygen saturation.

Clinical course

Having undergone inpatient treatment as described above, wheezing was eliminated in five days on average in both the BIS and PSL groups with five days of steroid use on average (**table 2**). Hypoxemia occurred in eight patients in the BIS group and in six patients in the PSL group. The number of days of oxygen use tended to be fewer in the BIS group, although the difference was not statistically significant (BIS group n = 4.2 days, PSL group n = 6.3 days, p > .05).

Table 2 *- Course after hospitalization.*

	BIS (n = 30)	**PSL (n = 20)**	
Days of wheezing detected	5 ± 0	5 ± 1	
Days of steroid therapy	5 ± 0	5 ± 0	
Patients with desaturation	8	6	
Days of oxygen required	2 ± 1	3 ± 1	
Serum cortisol at admission (μg/dL)	15.0 ± 2.2	17.2 ± 2.1	
Serum cortisol at re-examination	17.0 ± 1.2	10.9 ± 1.5*	*$p = 0.0036$
Days after admission for re-examination	4 ± 0	4 ± 0	

In both groups, wheezing disappeared in 5 days on average with 5 days of steroid use on average. In the initial 4 days (mean) of hospitalization, the serum cortisol level remained unchanged in the BIS group, while it decreased significantly in the PSL group.

Laboratory data

Serum cortisol levels in the BIS and PSL groups at the time of admission were 15.0 μg/dL and 17.2 μg/dL (p > .05), respectively. However, serum levels on the fourth day of hospitalization were 17.0 μg/dL and 10.9 μg/dL, with significant suppression in the PSL group. Adverse events did not occur in either group.

Discussion

Treatment of children less than 3 years of age with mild exacerbations of asthma using high-dose nebulized budesonide therapy is as effective as systemic steroid therapy. Furthermore, serum cortisol suppression, which was observed in patients treated with systemic steroids, did not occur in patients treated with high-dose nebulized budesonide therapy. This suggests that high-dose nebulized budesonide therapy results in the same therapeutic outcome as systemic steroid therapy, without some of the systemic effects of steroid administration.

Making diagnosis of asthma in children less than 3 years of age is difficult since recurrent wheezing is often detected with vi-

ral infections and lower respiratory infections in this age. Our study included children with recurrent wheezing more than 3 episodes regardless of the existence of respiratory infections. Six of all received antibiotics therapy due to acute lower respiratory infections with increased white blood cell count and elevated C-reactive protein which indicated a bacterial infection. A presence of lower respiratory infection does not exclude a diagnosis of asthma exacerbation with recurrent wheezing children. In fact, the characteristic pathologic features of asthma such as thickening of the bronchial epithelial reticular basement membrane and eosinophilic airway inflammation were reported to be seen even in a part of children with recurrent wheezing at 3 years of age (4). Since steroid therapy is not effective to acute bronchiolitis (5), we carefully recruited asthma children less than 3 years of age. To identify which children will have persistent asthma, various predictions of persistent asthma in younger children have been proposed. According to the Japanese asthma diagnostic criteria, the recurrent wheezing more than 3 episodes is major criteria to identify persistent asthma in children less than 3 years of age. This highlights the importance of early intervention in younger children with asthma for maintaining their lung function. However, there is a limitation to include children with repeated acute bronchiolitis. The Asthma Predictive index (API) is another prediction to identify persistent asthma (6). A positive API at age 3 has a sensitivity of 17-19% and specificity of 99-100% for asthma between ages 6-8 years (7,8). Our study included 44 of 51 positive API children (80%). Remaining seven children with negative API had repeated wheezing episodes apart from colds, but had no family history of parental asthma, no medical history of eczema or allergic rhinitis in previous examinations. These children had possibility to be underestimated with eczema because of less chance to visit medical institutions and to be later diagnosed with eczema or allergic rhinitis until their 3-years-old birthday.

While long-term systemic steroid administration has been known to cause some adverse reactions, such as growth suppression, bone metabolism disorders, and adrenal gland dysfunction, few side effects have been identified in short-term systemic steroid therapy. Recent reports have shown that symptoms of anxiety, depression and deterioration of memory emerge after short-term systemic steroid therapy (9,10), and bone metabolism disorders occur when short-term systemic steroid therapy is given repeatedly (11). Caution must be taken regarding systemic side effects even when short-term systemic steroid therapy is given.

Inhaled steroid therapy has been considered to have fewer systemic effects and to be safer than systemic steroid therapy. However, some reports have shown that bone metabolism disorders and growth suppression occur in long-term moderate- to high-dose inhaled steroid therapy (12,13), and that the growth tends to be suppressed when short-term (up to 10 days) high-dose inhaled steroid therapy is repeated (7 times on average in one year) (14). Therefore, the safety of inhaled steroids for long-term and repeated treatment has not been determined.

In the present study, the number of days of high-dose nebulized budesonide therapy was as long as nine days, and was five days on average. Only 10 children in both groups (20%) underwent repeated systemic steroid therapy or high-dose nebulized budesonide therapy after participation in the study with a mean of two treatments per child, indicating that a very low dose of steroid was required per child compared to previous studies. Possible reasons for this difference include that, in the report from Ducharme et al., high-dose nebulized budesonide therapy may have been given to some children who were not in need, because the treatment was chosen at guardians' discretion (10). If high-dose nebulized budesonide administration was used at the physicians' discretion based on the asthma exacerbation, as done in the present study, the number of treatments and days of treatment may be minimized, and thus the risk of growth suppression is likely to be further reduced.

Serum cortisol suppression, which was observed in patients treated with systemic steroid therapy, did not occur in patients treated with high-dose nebulized budesonide therapy, suggesting that high-dose nebulized budesonide therapy for approximately five days is unlikely to have systemic effects. Furthermore, no adverse event was noted during this study, suggesting that administration of high-dose nebulized budesonide therapy is safe. Nevertheless, peri-oral pruritus, pharyngeal pain, and hoarseness are possible in patients receiving high-dose inhaled steroid therapy as reported previously. In general, inhalation of fluticasone is considered to induce a stronger reaction and reports with budesonide are limited (15), suggesting possible differences in biological activity among different steroids.

In addition to anti-inflammatory effects via gene expression, inhaled steroids have recently been reported to exert an early anti-inflammatory effect that is not mediated by gene expression. Improved clinical symptoms have been noted as early as four hours after inhalation in reports on high-dose inhaled steroid therapy for asthma exacerbation in infants and toddlers (16), and high-dose inhaled steroid therapy improves respiratory function and clinical symptoms more rapidly than systemic steroid therapy (17,18) (**table 3**).

It has also been reported that, in patients with asthma undergoing high-dose inhaled steroid therapy, airway blood flow is reduced to about half at 30 minutes after inhalation and returns to the original level about 90 minutes after inhalation (19), suggesting that such changes may contribute to the improvement in airway narrowing and decrease in airway secretions. In children who developed hypoxemia in the present study, those in the BIS group had more rapid improvement of hypoxemia than those in the PSL group, although the difference was not signif-

Table 3 *- Reports on high-dose inhaled budesonide and systemic steroid therapy for acute exacerbation of moderate to severe infantile asthma.*

	No of patients (ages)		Protocol	Results
Volovitz et al. (1998)	22 (6-16 yr)	Moderately severe BA	Initial: pMDI BUD 1.6 mg vs. PDN 2 mg/kg After 1st day: reducing dose for 1 wk	BUD group: Clinical symptoms up to 4 hours after the start of treatment were improved earlier. PDN group: The serum cortisol level decreased in the first and third weeks after the start of treatment. No difference in the degree of respiratory disorders and the peak flow value
Matthews et al. (1999)	46 (5-16 yr)	Severe BA	Initial: neb BUD 2 mg x 3 times daily vs. PDN 2 mg / kg at immediately and 24 h After 1st day: pMDI BUD 0.8 mg for 24 d	BUD group: Greater improvement in 1-second volume 24 hours after the start of treatment
Sano et al. (2000)	71 (3-24 mo)	Acute wheeze with dyspnea	Initial: neb BUD 0.25 mg x 4 / d + HDC 40 mg/kg iv vs. HDC 40 mg/kg Continued till discharge	BUD group: Greater improvements in clinical symptoms after 12 hours after starting the treatment and the respiratory rate 24 hours after starting the treatment Treatment period was shorter (BUD 66.4h vs. HDC 93hr)

BA: bronchial asthma; pMDI: pressurized metered-dose inhaler; BUD: budesonide; PDN: prednisolone; HDC: hydrocortisone

icant, suggesting that high-dose nebulized budesonide therapy improves airway contraction/airway inflammation more rapidly. Unfortunately, airway inflammation could not be evaluated by objective means such as spirometry or exhaled nitric oxide measurement, because of the patients' age.

In children less than 3 years of age, tests to assess chronic airway inflammation and airway hyper-responsivity required to establish the diagnosis of asthma can be performed in only a few institutions. Therefore, asthma tends to be underdiagnosed in typical medical facilities. As a consequence, appropriate steroid therapy may not be given during asthma exacerbations in young children. It has been reported that infants with repeated past episodes of untreated wheezing have pulmonary impairment that persists until young childhood or even until adulthood (20-22). Although the side effects of inhaled steroids remain an issue, long-term preservation of pulmonary function can be expected from high-dose nebulized budesonide therapy given to improve airway inflammation and constriction during asthma exacerbations in infants, as in the present study. Therefore, clinicians should not hesitate to use this therapeutic option.

There are a number of acknowledged limitations to this study. A double blind study design was not used, thus the type of treatment was identifiable from the appearance of the drug. Biases among medical staff and parents cannot be ruled out. Second, making a confident diagnosis of asthma in children less than 3 years of age is difficult because wheezing is often detected with viral infections and lower respiratory infections in younger children. For this reason, we recruited children at high risk of developing asthma with repeated wheezing according to the guideline proposed by the Japanese Association of Allergology, but the possibility still remains to have included children without asthma. Third, while suppressed serum cortisol levels were observed in the PSL group, the suppression was not pathologic, and we could not determine if adrenal function was actually suppressed, because an adrenal function loading test was not conducted. Symptoms of adrenal insufficiency such as low activity, gastrointestinal symptoms, unexplained fever, or hypoglycemic symptom, were not observed in either group. Therefore, both high-dose nebulized steroid therapy and systemic steroid therapy for five days were considered unlikely to lead to immediate adrenal insufficiency.

Conclusion

High-dose inhaled steroid therapy was at least not inferior to systemic steroid therapy in therapeutic efficacy for children less than 3 years of age with mild exacerbations of asthma. More-

over, unlike in patients who received systemic steroid therapy, suppression of serum cortisol levels was not observed in patients receiving high-dose nebulized steroid therapy, suggesting that it has reduced systemic effects.

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Esophageal dysfunction and immunological changes induced by grass sublingual immunotherapy

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Key words
sublingual immunotherapy; Phleum; esophageal diseases; Immunoglobulin IgE

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Summary
Sublingual immunotherapy frequently causes local oropharyngeal adverse events which are usually of mild severity, and tend to be self-limited and disappear within the first weeks of therapy. The mechanism of action involves changes in the specific humoral response to allergens, with increases in allergen-specific immunoglobulin G4 (IgG4) and blunting of the seasonal increase in allergen-specific IgE.
We describe the case of a 25-year-old man diagnosed with grass pollen induced allergic rhinoconjunctivitis, who was treated with a lyophilisate of Phleum pratense *by sublingual route. After 5 weeks of therapy he developed repeatedly intense symptoms of esophageal dysfunction immediately after the administration. Symptoms recurred every day, subsided in some hours without treatment and disappeared with the termination of therapy. The episode coincided with a marked elevation of total and specific IgE. The immunological changes gradually declined during the three years of follow up.*
The reported case suggests the need to evaluate the role of the immunological changes detected after the first weeks of sublingual therapy with Phleum pratense*, in the induction of esophageal disorders.*

Introduction

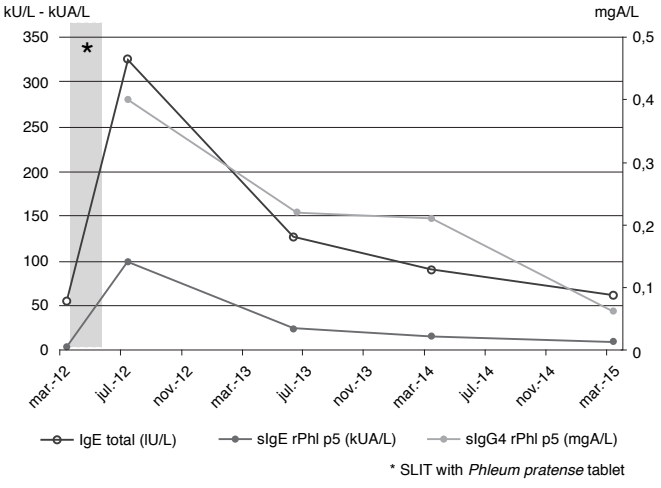
Sublingual allergen immunotherapy (SLIT) shows a high tolerability, allowing self-administration at home. A significant percentage of patients show minor local side effects (oral pruritus and/or edema, throat irritation) which usually disappear within a few days of treatment (1). In the two forms of SLIT allergen preparations, drops or tablets, the aim is sublingual absorption. Therefore, patients are instructed to avoid swallowing it.
Esophageal involvement is not an expected consequence of SLIT and there is no scientific evidence linking esophageal dysfunction (such as reflux or esophagitis), with SLIT (2). However, there are several reports of eosinophilic esophagitis (EoE) in children after oral desensitization with food allergens (milk, egg) (3,4), and there are two cases of early EoE after a month of pollen SLIT. The first case, reported in 2013, was related to the administration of liquid SLIT containing pollens (5). The second case occurred in a patient receiving sublingual tablets with timothy grass (6). In both cases EoE was confirmed by biopsy and disappeared after SLIT was withdrawn.
The immunological effects of SLIT include the capture by the mucosal dendritic cells and the presentation to T cells, which bias the response to a TH1 profile, away from a pro-IgE TH2 profile. However, during the first month of therapy the sublin-

gual administration of a *Phleum pratense* tablet induces a TH2 immune response characterized by increased levels of allergen specific IgE, IL-4, IL-5 and IgG4. This phenomenon is followed by a TH1 response with reduced levels of IL-4, IL-5 and IgE; and increase of IgG4 and a CD4+ cell response (7).
We describe a case of marked immunological changes occurring in a patient treated with a sublingual grass pollen tablet who developed esophageal dysfunction symptoms.

Case report

A 25-year-old man was referred to our allergy clinic with grass pollen moderate-severe allergic rhinoconjunctivitis refractory to standard therapy with antihistamines and intranasal corticosteroids. The patient's medical history included seasonal allergic rhinoconjunctivitis treated for 2 years with subcutaneous immunotherapy (*Lolium perenne, Phleum pratense* and *Chenopodium album*) in 2007, without significant improvement. He denied any history of food allergy, gastrointestinal symptoms or esophageal pain. The initial allergy evaluation was positive for grass pollen (specific IgE to *Phleum pratense*: 5.41 kUA/L) with a normal serum level of total IgE (54.7 kU/L). Sensitization to dust mites, molds, animal dander, other pollens and foods was ruled out by skin prick-tests and/or specific IgE (*Derm pteronyssinus*: 0.16 kUA/L, *Derm. farinae*: 0.13 kUA/L, dog dander: 0.18 kUA/L, cat dander: 0.14 kUA/L, olive pollen: 0.67 kUA/L, peach lipid transfer protein 0.48 KU/L, phleum profilin: 0.03 kU/L).
Treatment with a daily oral lyophilisate (tablet) of grass pollen from Timothy (*Phleum pratense*), 75,000 SQ by sublingual administration (GRAZAX®, ALK) was initiated in April 2012 without immediate complications after the first doses. Five weeks later he turned up referring a significant improvement of the nasal symptoms and no need of antihistamines. However, he complained of an intense esophageal burning sensation and epigastric pain increasing by trunk flexion immediately after the intake of the tablet and persisted for about 30 minutes until gradually calming down. These symptoms appeared after four weeks of therapy and reappeared the following four days after the tablet administration. SLIT was discontinued and therapy with omeprazole 20 mg/day initiated with a rapid resolution of symptoms.
At the moment of SLIT discontinuation, total and specific *Phleum pratense* IgE were markedly increased (**figure 1**). The patient was monitored during three years observing a gradual decrease in the levels of specific IgE and IgG4. Symptoms of allergic rhinoconjunctivitis were milder and well controlled with few doses of antihistamines during the following three grass pollination seasons. No gastric or esophageal symptoms reappeared.

Figure 1 *- Changes in total IgE and IgG4.*



Discussion

In the present case, symptoms of esophageal dysfunction were related to the sublingual administration of a lyophilisate of *Phleum pratense* after four weeks of treatment. This episode coincided with a marked elevation of total and specific IgE. Symptoms disappeared with the termination of therapy and the immunological changes gradually declined during the three years of follow up. We didn't restart grass tablet SLIT to ethical considerations.
The role of the immune response induced during the first month of SLIT on the esophagus, provoking an acute esophageal inflammation, is not determined. The initial immunological effects, with increased levels of allergen specific IgE and changes in the cellular response might be related to the frequent local application site reactions. As IL-5 is a key mediator in eosinophil activation we hypothesize that allergen exposure may induce esophageal symptoms through the effect of IL-5 on eosinophil activation.
The two published cases of EoE related to the administration of SLIT shows some coincidences with our case. The patients, without previous esophageal or gastric disease, reported esophageal symptoms four weeks after starting SLIT (5,6). Symptoms were mild and resolved after stopping therapy without further need of treatment. No immediate local oropharyngeal adverse events were reported.
In the case we report, the rapid clinical improvement after discontinuation of SLIT and starting a proton pump inhibitor (PPI), avoided the performance of an endoscopy with biopsy which could confirm the diagnosis of EoE. The favorable response should not support the diagnosis of a peptic disorder because PPI have anti-inflammatory effects (8). Two recent studies

showed that PPI inhibit TH2 cytokine-stimulated secretion of eotaxin-3, that this is the primary eosinophilic chemoattractant in EoE, in the esophageal squamous cell (9,10). Thus, therapy with PPI could explain the rapid improvement in our patient.
The esophageal symptoms coincided with the highest levels of specific IgE and probably, IL-5. However, although the esophageal dysfunction disappeared with the discontinuation of SLIT, the levels of total IgE, specific IgE rPhl p5 and specific IgG4 rPhl p5 decreased gradually to previous levels in three years.
We recommend discontinuing therapy in patients receiving SLIT who complain of esophageal symptoms. Analytical and histopathological studies should be performed in order to investigate the role of the specific immunological changes in the induction of esophageal inflammatory processes.

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Hemophagocytic Lymphohistiocytosis mimics many common conditions: case series and review of literature

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Key words

Hemophagocytosis; hemophagocytic lymphohistiocytosis; macrophage activation syndrome; systemic inflammatory response syndrome; T-cell dysregulation; immune regulation

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Summary

Introduction. *Hemophagocytic lymphohistiocytosis (HLH), a rare but potentially fatal disease, is characterized by excessive immune activation and cytokine release which stimulates bone marrow macrophages to engulf hematopoietic cells. HLH could be secondary to infections: viral, fungal, and bacterial; malignancies and autoimmune diseases. The diagnosis of HLH is usually delayed due to the presence of non-specific symptoms at presentation. This delay contributes to increased mortality.* ***Cases and review***. *We present the case of 4 patients who presented with subjective fevers and extreme fatigue. Patients all had systemic inflammatory response syndrome (SIRS). All patients were initially managed as in sepsis from an underlying infection. All unfortunately progressed to multiple organs dysfunction and died. The underlying causes for HLH in the patients were considered to be: HIV/AIDS, T cell lymphoma, histoplasmosis and juvenile rheumatoid arthritis. We have also included a brief review of the literature on HLH highlighting the treatment and outcomes of patients in case series; and the many conditions which can trigger HLH.* ***Conclusion***. *Patients with HLH usually share various non-specific symptoms, such as fever and malaise, with patients across a wide spectrum of conditions: from bacterial sepsis to malignancies. Since early suspicion and diagnosis is critical to prompt therapy and improved mortality, including HLH as a possible cause of fever particularly in patients with prolonged fever of unknown origin and cytopenias will be crucial.*

Introduction

Hemophagocytic lymphohistiocytosis (HLH), a rare but potentially fatal disease, is characterized by persistent immune activation and cytokine release which stimulates bone marrow (BM) macrophages to engulf hematopoietic cells. Over the past three decades there has been extensive research into the causes of HLH, its diagnosis, and its optimal management. This, in addition to the publication of the HLH-1994 and HLH-2004 diagnostic criteria and treatment protocols has led to an improvement in the prognosis of HLH (1). Since the publication of the HLH-94 protocol, mortality in primary HLH has been shown to improve from about 100% in a few months to about 50% over a median follow-up of six years (2). However, the prognosis of secondary HLH, which is more common in adults, remains grave (3).

HLH could be primary or secondary (1,4). Primary or familial hemophagocytic lymphohistiocytosis (FHL) has underlying genetic mutations inherited in either an autosomal recessive pattern (perforin [PRF1], MUNC13-4, syntaxin 11 [STX11], STXBP2, and RAB27A genes) or X-linked pattern (SH2D1A

and XIAP/BIRC4 genes) (5-13). Secondary HLH (sHLH) has been described in patients with HIV/AIDS, malignancies such as lymphomas, viral infections, and mycobacterial infections. Autoimmune disorders, such as systemic lupus erythematosus, multiple sclerosis and myasthenia gravis, could also trigger HLH in which case it is usually referred to as macrophage activation syndrome (14). Although adults usually have sHLH, 10-15% of adults with HLH have an underlying genetic mutation, commonly in the perforin gene (15).

Herein, we report the clinical course of four adult patients who were diagnosed as having HLH in our center with emphasis on the similarities between their clinical presentations and features to facilitate increased suspicion and prompt diagnosis. Finally, we include a concise review of literature emphasizing common conditions associated with sHLH and outcomes of the patients reported.

Methods

Case Series: We present briefly a summary of the clinical course of four adult patients.

Review of the Literature: Using the search terms "lymphohistiocytosis," "hemophagocytosis," "HLH," "MAS," alone and in all combinations, we identified reports of HLH in PubMed from 2004 through March 2015. We excluded case reports of single patients and included only case series of adult patients.

Case series

All four patients (**table 1**), aged 20-60 yrs, initially presented with subjective fevers and extreme fatigue. 3 of the 4 were otherwise healthy patients with no significant comorbidity prior to current illness. Patient 1 had underlying HIV on highly active antiretroviral therapy (most recent CD4: 218, viral load < 20 copies/ml). On presentation, 3 of these 4 patients met SIRS criteria, most commonly being febrile and tachycardic and/or hypotensive. All were anemic and thrombocytopenic. Patient 1 was also leukopenic. All of these patients were initially treated with broad-spectrum empiric antibiotics for presumed sepsis although multiple bacterial cultures subsequently returned negative in all of them except in Patient 3, who later went on to develop Enterobacter aerogenes bacteremia weeks after the diagnosis of HLH. Due to persistent fevers, despite antibacterial regimen, and chronicity of symptoms, other etiology such as HIV/AIDS and tuberculosis were explored with an extensive workup for atypical bacteria, viruses, fungi and parasites given the hospital's location in an area with high incidence of HIV and large immigrant population. Ferritin levels were subsequently checked which was remarkably greater than 1000 ng/ml in all patients, and in Patients 1 and 4 peaked at 55,269 and 87,708 ng/ml respectively. Elevated ferritin and worsening cytopenias triggered bone marrow aspiration that led to the diagnosis of HLH. 3 of the 4 had hemophagocytes in the bone marrow but all met criteria for HLH as defined in the HLH-2004 guidelines. The underlying disease potentially causing HLH was later considered to be HIV/AIDS in Patient 1, hepatosplenic T-cell lymphoma in Patient 2, and histoplasmosis in Patient 3. In Patient 4, who was 20 years old at time of diagnosis, the underlying etiology was considered to be juvenile rheumatoid arthritis. A few months after Patient 4 died, however, brother presented with similar illness. Genetic testing was however not pursued as per family's wishes.

Patients 1 and 2 both received 1 cycle of chemotherapy. Patients 3 and 4 were considered too sick at the time of HLH diagnosis with the risk of chemotherapy outweighing the perceived benefit. Patient 1, who was HIV-positive, initially showed improvement in clinical status and ferritin level dropped significantly by 75% while platelet count and leucopenia improved within the first 2 weeks of treatment. Patient however defaulted and was readmitted about 3 months after HLH diagnosis with herpes encephalitis. HLH relapsed and patient progressed to multi-organ failure (MODS) within 4 months of diagnosis. Patient 2 however continued to deteriorate despite chemotherapy. He progressed to multi-organ failure within 8 weeks of diagnosis. Patient 3 was initially placed on empirical anti-tuberculous regimen due to immigration from a tuberculosis-endemic area, and the presence of hilar lymphadenopathy and apical nodules on chest radiography. Repeated acid-fast bacilli stain of bronchial washings and mycology cultures of bone marrow biopsy specimen were however negative. Computed tomography imaging of the chest however revealed widespread granulomas: including at the apices and bases of the right lung with suggestion of histoplasmosis. Patient was placed on fluconazole. Patient 4 received plasmapheresis and pulsed steroids. Cytopenias and inflammatory markers continued to worsen in both patient 3 and 4. Fever initially resolved in both patients but both developed disseminated intravascular coagulation shortly after treatment was started with consequent fatality.

Figure 1 *- Bone marrow biopsy (showing macrophage engulfing hematopoietic cells).*

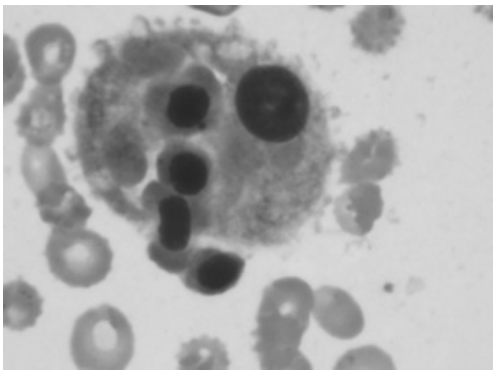

Table 1 *- Demographic characteristics, clinical manifestations, laboratory results, and clinical outcomes of the 4 patients with hemophagocystic lymphohistiocytosis.*

	Patient 1	**2**	**3**	**4**
Age, years	45	39	60	20
Gender	female	male	male	male
Duration of illness prior to presentation	2 weeks	2 days	1 month	3 months
HLH Diagnostic criteria				
i. Fever (> 38.5 °C):	+	+	+	+
ii. Splenomegaly	+	+	-	-
iii. Cytopenia (affecting ≥ 2 of 3 lineages in peripheral blood)	+	+	+	+
Neutropenia, absolute neutrophil count < 1000/L	+	+	+	-
Anemia, Hb < 9g/dL	+	+	+	+
Thrombocytopenia, Platelet < 100/L	+	+	+	+
iv. Hypertriglyceridemia, TG (fasting ≥ 265 mg/dL) or hypofibrinogenemia, FBRN (≤ 150 mg/dL)	+ (↑TG)	-	'+ (↑TG)	'+ (↑TG and ↓FBRN)
v. Low or absent NK cells activity	not tested	not tested	not tested	not tested
vi. Hyperferritinemia (≥ 500 µg/L)	+	+	+	+
Ferritin, peak (ng/mL)	55269	2949	4958	87708
vii. High levels of sCD25, a.k.a IL-2Rα (≥ 2400 U/mL)	not tested	not tested	not tested	not tested
viii. Hemophagocytosis in the bone marrow, spleen or lymph nodes	+	+	-	+
Initial presentation				
Tachycardia (HR > 100)	+	+	+	+
Tachypnea (RR > 20)	-	-	+	-
Other features	lymphadenopathy	jaundice, hepatomegaly	mediastinal and hilar lymphadenopathy	lymphadenopathy, skin rash, altered mental status
Underlying and Associated diseases	HIV/AIDS	T cell lymphoma	granulomatous disease likely Histoplasmosis	MAI pneumonia, juvenile rheumatoid arthritis
Other Laboratory results				
LDH (U/L)	564	2354	660	2769
ALP (U/L); initial	238	295	145	113
ALP (U/L); peak	2719	563	358	1049
ANA panel	-	-	-	+
EBV IgM or IgG	+: IgG	+: IgG	no	+: IgM
CMV IgM or IgG	+: IgG	+: IgM and IgG	+: IgG	not tested
Treatment regimen	HLH-2004	hyper CVAD, ICE, ESHAP	RIPE	pulsed steroids and plasmapheresis
Clinical course and outcome				
DIC	-	-	+	+
Cause of death	multi-organ failure	multi-organ failure	multi-organ failure	diffuse cerebral edema and SAH
Survival (days): from diagnosis of HLH	147	54	79	27

MAI: Mycobacterium Avium Intracellulare; ALP: alkaline phosphatase; DIC: disseminated intravascular coagulation; Hyper CVAD: Cyclophosphamide, Vincristine, Adriamycin, Dexamethasone; ICE: Ifosfamide, Carboplatin, Etoposide; ESHAP: Etoposide, methylprednisone, Ara-C (cytarabine); RIPE: Rifampin, Isoniazid, Pyrazinamide, Ethambutol; SAH: subarachnoid hemorrhage; Hb: hemoglobin; HIV: Human immunodeficiency virus infection; IVIG: intravenous immunoglobulin; LDH: lactate dehydrogenase.

Case reviews and associated diseases

35 case series which included at least 2 adult patients were identified (**table 2**) (16-43,4,44,45,14,46-49). **Table 2** shows the details of these included case reviews, including treatment regimen and patient outcomes. These case series included a range of 2 to 52 adult patients. Any condition that can trigger an inflammatory reaction, infectious or non-infectious, can cause HLH (**table 3**). (50-54) HLH-2004 (or -94) protocol was used in less than half of the cases. However, other chemotherapeutic regimen, such as Cyclophosphamide-Adriamycin-Vincristine-Prednisone (CHOP) were used. This could be due to practice variations and providers experience using certain regimen, or as was in our case, the frailty of the patients such that the patients were considered too weak to tolerate the chemotherapy regimen recommended. Multiple other therapies directed at the underlying trigger for HLH were used. These included rituximab in most cases where EBV was suspected, plasmapheresis, IVIG, pulsed steroids and antiviral agents. Some patients also had stem cell transplantation (SCT). Mortality ranged from 0 to a 100% over the time period these patients were followed. Mode was 100% mortality and mean was 67%. Remarkably, patients who had SCT had improved survival. Time to death ranged from 5 hours after diagnosis to a patient who was still alive 15 years after SCT for FHL.

Table 2 *- Summary of the case series included in review[1].*

Article	Number of patients reported	Suspected etiology of HLH	Chemotherapeutic regimen and dosage; Adjunct therapy	Mortality rate if reported	Time to death if reported
Park et al[4]	23	EBV, Hepatitis A	HLH-94 or 2004 (dexamethasone, etoposide, and cyclosporine) protocol; corticosteroids, cyclosporine; HSCT	74% in 6 months	Median: 41 days
Tseng et al[5]	96	Viral infections: e.g. CMV, mycobacterial, bacterial: e.g. Aeromonas, and fungi: e.g. cryptococcus; Nosocomial- e.g. burkholderia). Still's disease, SLE, livedoid vasculitis, Sjogren's syndrome, and psoriasis. Hematology / oncology disorders	IVIG, corticosteroids, etoposide	63% 30-day mortality	-
Sieni E et al[6]	11	FHL; Infectious mononucleosis-like illness, Non-Hodgkins lymphoma, neurosarcoid, Herpes	HLH-94 or HLH-2004 protocols; autologous or allogeneic SCT	64% within 15 years.	ranged from early progressive death shortly after diagnosis -to 10 yrs. 1 patient identified as 'cured' post-SCT
Abe et al[18]	5	Primary EBV; EBV reactivation	chemotherapy with or without etoposide; plasmapheresis (for patient with severe symptoms)	40% mortality over 30 months	-
Argyraki et al[19]	3	EBV; MSSA-Infective endocarditis	corticosteroids, IVIG; intravenous cloxacillin for MSSA endocarditis	0%. All patients alive at 12 months post-diagnosis	-
Ben Dhaou Hmaidi et al[20]	4	Adult-onset Still's disease; Sjogren syndrome; severe sepsis	corticosteroids; other immunosuppressant therapy	50%	unclear
Berry et al[21]	2	EBV infection	IVIG, corticosteroids; antivirals (famciclovir, acyclovir)	100% within a week of admission	5 days

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Article	Number of patients reported	Suspected etiology of HLH	Chemotherapeutic regimen and dosage; Adjunct therapy	Mortality rate if reported	Time to death if reported
Besset et al[22]	9	not reported	etoposide-containing regimen (specifics unknown)	33% died in ICU; 5 (56%) died in hosp	mean ICU LOS: 7 days; mean LOS: 21 days
Bohne et al[23]	2	EBV; Influenza A/H1N1 infection, underlying XLP-1 (SH2D1A) mutation; cerebral aspergillosis	HLH-2004 +rituximab	100%	Death shortly after diagnosis despite treatment
Buyse et al[24]	56	Hematology/oncology disorders (Castleman's dx, B-cell lymphoma, other); Infections: non-viral and viral; 68% of patients had underlying immune-deficiencies	Etoposide, IVIG, corticosteroids	52% while in the hospital	(median hospital LOS: 23.5 [11.2-41.7] days)
Chellapandian et al[25]	42	EBV	HLH-2004 + rituximab; IVIG; antivirals (ganciclovir, acyclovir); allogeneic SCT	38% died within 900 days	-
Fox et al[27]	3	EBV; Hodgkins Lymphoma	-IVIG, rituximab, ganciclovir; immunosuppressant: cyclosporin A, coorticosteroid; etoposide	67% within 8 weeks of follow up	-
Fukunaga et al[28]	2	malignancy	low-dose etoposide and vincristine plus prednisolone	0%	still alive 1008 and 232 days after transplantation
Gold et al[29]	2	Rheumatoid arthritis.	corticosteroid, cyclosporine, etanercept, and plasmapheresis; intrathecal methotrexate.	50% within 90-day follow up	24 days
Hu et al[30]	15	Infection- MRSA, CMV, EBV; Autoimmune disease; Malignant lymphoma	COP (HLH-2004 protocol used as salvage therapy in 2 patients); allogeneic-HSCT in a patient with lymphoma	33% at 1-year	-
Kelesidis et al[31]	4	EBV reactivation, Chronic Granulomatous Disease	HLH-2004+rituximab; Ganciclovir, IVIG, cyclosporine	75%	not stated
Lecronier et al[32]	17	Q fever, Mediterrnean spotted fever	Doxycycline; +/- levofloxacin; IVIG, hydroxychloroquine, corticosteroids	0%	all patients recovered
Loa et al[33]	2	following kidney transplant for FSGS; disseminated histoplasmosis	IV liposomal Amphotericin B, oral itraconazole (for 12 months)	0% in 10-month follow up	-
Machaczka et al[34]	8	CLL, Multiple Myeloma, Waldenstroms, T-cell lymphoma, Hodgkin's Lymphoma	IVIG and corticosteroids; HLH-94 protocol	88% over 13 months of follow up	1 week to 13 months
Mayson et al[35]	2	EBV, T-cell lymphoma	HLH 2004 protocol; IVIG, corticosteroids	0% at 3 weeks post-HLH diagnosis	-

Article	Number of patients reported	Suspected etiology of HLH	Chemotherapeutic regimen and dosage; Adjunct therapy	Mortality rate if reported	Time to death if reported
Miguel et al[36]	2	CMV infection in patient on azathioprine for Crohn's disease	IVIG, corticosteroids, antivirals (gancoclovir, valganciclovir)	0% over 8-and 18-month follow up respectively	-
Mitra et al[37]	3	FHL, tuberculosis, diffuse non-Hodgkin T-cell lymphoma	patient 1: only supportive treatment; patient 2: four-drug anti-mycobacterial for 6 months; patient 3: CHOP protocol	67% over 30-days	median: 14 days
Nieto-Ríos et al[38]	2	Disseminated histoplasmosis; kidney transplant	Amphotericin B and itraconazole; immunosuppresant therapy: alemtuzumab induction and maintenance with mycophenolate and cyclosporine / tacrolimus	50% within 3 days	-
Okabe et al[39]	3	EBV, sarcoidosis	IVIG. Others: infliximab, daclizumab, dexamethasone, and cyclosporine	100% within 12 days	-
Premaratna et al[40]	2	Rickettsial infections: Orientia tsutsugamushi and Rickettsia conorii	Doxycycline	0%	(Hematological recovery in 72-96 hrs of initiating treatment)
Rajagopala et al[41]	10	EBV, leishmanaia, leptospirosis, Parvo B19, SLE, tuberculosis, invasive mucormycosis	HLH 2004 protocol, corticosteroid, IVIG; Other: antiviral, antimalarial, antimycobacterial TB, amphotericin	70% died in ICU; 80% in-hospital	ICU LOS (5 hrs to 15 days); hosp LOS: 2-21 days
Raschke et al[42]	3	suspected bacterial infection	HLH 2004 protocol	100% mortality	
Re et al[43]	2	Human herpesvirus 8 (HHV-8) / Kaposi sarcoma-associated herpesvirus (KSHV); CMV	IVIG, corticosteroids, antivirals (gancoclovir, acyclovir)	100%	unknown
Shabbir et al[44]	18	haematological malignancies, post-autologous stem cell transplant, infection, rheumatologic illness, sickle cell disease, post-orthotopic liver transplant	Etoposide, IVIG, cyclophosphamide. Immunosuppressants: corticosteroids +/- cyclosporine	72%	median survival: 35 days
Soyama et al[45]	2	chronic hepatitis C infection with HCC, now post liver transplantation; chronic hepatitis B with HCC	IVIG, corticosteroid; GM-CSF, entacavir	100% mortality within 5 months of diagnosis	-
Takeoka et al[46]	2	EBV; T cell lymphoma	patient 1: dexamethasone, acyclovir and etoposide; patient 2: CHOP regimen	100% within 4 months	mean time: 3 months
Ueda et al[47]	16	SLE	intravenous cyclophosphamide. Pulsed corticosteroid, IVIG, plasmapheresis, azathioprine / tacrolimus / rituximab	13%	unknown

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Article	Number of patients reported	Suspected etiology of HLH	Chemotherapeutic regimen and dosage; Adjunct therapy	Mortality rate if reported	Time to death if reported
van Langenberg et al[48]	2	CMV infection in patient on azathioprine for inflammatory bowel disease	Ganciclovir; hydrocortisone	0%	patient 1: remains well after 5 year follow up; patient 2: remains well at 16 month follow up
Young et al[49]	4	CMV infection; Hantavirus pulmonary infection; pneumonia with Acinetobacter baumanii	IVIG, corticosteroid, cyclosporine	66% in 47 days	-
Yu JT et al[50]	30	T-cell and B-cell lymphoma	CHOP/CHOP-like +/- Rituximab (for B-cell). Other: hyper-CVAD, ESHAP, steroid only); Allogeneic SCT in three patients	93%	median overall: 231 days. 330 days (B-cell); 93 days (T-cell). 2 patients with T-cell lymphoma and SCT had complete disease remission

[1]Some studies reported only median age. Such studies noted; SCT: stem cell transplantation; LOS: length of stay; HCC: hepatocellular carcinoma; G-CSF: granulocyte colony stimulating factor; CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone.

Pathogenesis

The key pathogenic feature of HLH is hypercytokinemia. Foreign materials, such as organisms and tumors, activate cytotoxic T lymphocytes (CTL) and natural killer cells (NK) cells. When activated, these cells form secretory lysosomes, which contain perforin and granzyme B. Perforin makes pores in the surface of target cells and granzyme B enters the target cell to stimulate apoptosis. These activated CTL and NK cells also release numerous cytokines including interferon-gamma, Tumor necrosis factor (TNF)-alpha, interleukin-6 (IL-6), and colony-stimulating factor which stimulate bone marrow macrophages (55). In primary HLH, NK cells and CTL fail to eliminate their targets leading to sustained inflammatory response, continued activation of macrophages, and production of cytokines (1). In secondary HLH (sHLH), macrophages are activated as a result of an inciting immunogenic condition or agent. Hypercytokinemia leads to prolonged fevers, fatigue and they down regulate the expression of CD47 on the surface of hematopoietic cells. Self-recognition to prevent phagocytosis is regulated by the CD47-SIRPA (signal regulatory protein) interaction (56,57). Down regulation of CD 47 therefore leads to an imbalance favoring pro-phagocytic factors, such as calreticulin. Macrophages become activated and engulf erythrocytes, leukocytes, platelets, and their precursors in the marrow as well as other cells which might lack CD 47, such as lymphoma cells (58). This engulfment is responsible for cytopenias in patients with HLH.

Diagnostic criteria (1)

Diagnosis of HLH is made if 5 of the following 8 criteria are met or a molecular defect consistent with HLH is identified (1). Clinical criteria: fever (> 38.5 °C): usually present in > 90% of patients or splenomegaly. Laboratory criteria: cytopenia (affecting 2 of 3 lineages in peripheral blood); hypertriglyceridemia (fasting > 265 mg/dL) or hypofibrinogenemia (< 150 mg/dL); low or absent NK cells activity; hyperferritinemia (> 500 μg/L); and/or high levels of sCD25, also known as IL-2Rα (> 2400 U/mL) which indicates high T-lymphocyte activity. Histopathological criteria: hemophagocytosis in the bone marrow, spleen or lymph nodes with no evidence of malignancy. This is however not a prerequisite for diagnosis.
Other laboratory abnormalities that could be present in HLH include EBV IgM or IgG, positive ANA panel, proteinuria from hemophagocytes invading kidney, and high d-dimer (1).

Treatment regimen

In HLH-2004 guideline (1), the recommended regimen includes cyclosporine, etoposide, dexamethasone as well as intrathecal methotrexate. Other regimens that have been used include: CHOP, Cyclophosphamide-Vincristine-Prednisone (COP), Cyclophosphamide-Etoposide-Dexamethasone (CED). Medications like alemtuzumab, intravenous immunoglobulin (IVIG) and antithymocyte globulin have also been used.
SCT should be considered for patients with FHL, EBV-triggered HLH, or refractory HLH. Research has shown that selected patients, such as those with high fibrinogen, could also benefit from SCT.
Adjunctive treatment included in the HLH-2004 protocol include: rituximab (to be added to regimen if EBV-HLH since it kills CD-20 positive B-cells), splenectomy (in patients with massive splenomegaly), rFVIIa (in hyperfibrinogenemia and coagulopathy).

Table 3 *- Reported Diseases associated with HLH in Adults.*

Infectious		**Autoimmune diseases** [5,47]	**SLE**
Viral[5,18, 21, 23, 25, 31,36]	Epstein Barr Virus		Rheumatoid arthritis
	HIV/AIDS		Adult onset Stills disease
	Cytomegalovirus		
	Hepatitis A	Malignancies [34, 50]	
	Parvovirus B19	Primary	T-cell lymphoma
	Mumps virus		B-cell lymphoma
	Herpes Simplex virus		Hodgkin's lymphoma
	HHV-8		Chronic lymphocytic leukemia
	Dengue		Acute myeloblastic leukemia
	H1N1		Primary CNS lymphoma
	Parainfluenza		Anaplastic lymphoma
Fungal[5, 33, 38, 51-53]			Mantle cell lymphoma
	Mycobacterium spp		
	Histoplasmosis	Metastatic	Adenocarcinoma
	Aspergillosis		
	Mucormycosis	Transplant [38, 46]	liver, kidney
	Candidiasis		
Bacterial[5, 32, 40]		Other [5, 22, 26, 36, 39, 42, 48]	Sarcoidosis
	Rickettsia		Burns
	Yersiniosis		Severe trauma
	Brucellosis		DRESS
	Leptospirosis		Sezary syndrome
Protozoan[54, 55]			Inflammatory Bowel Disease
	Toxoplasmosis		Azathioprine
	Leishmaniasis		HAART
			Immune Reconstitution Syndrome

Prognosis

Prognosis depends on the underlying etiology. Nonetheless, the mortality of sHLH remains poor with most patients dying within 6 months of diagnosis. Death is commonly from multi-organ failure from either the complication of the HLH itself - including susceptibility to infections and bleeding from thrombocytopenia; or complication of the underlying disease - such as opportunistic infections in HIV; or from complications of treatment - commonly chemotherapy agents.

Favorable prognostic factors include: children, probably because etiology likely to be FHL; adults < 50 years, shorter time to treatment initiation, fevers subsiding within 3 days of diagnosis, low histiocytes in marrow, higher fibrinogen levels, absence of DIC or other coagulopathy and excellent baseline health.

Discussion

The overall evidence suggests that HLH is commonly triggered by infections. HLH could be considered a form of systemic inflammatory response syndrome (SIRS) (41). As with infection-triggered SIRS, early suspicion and prompt treatment is needed to avoid fatality. Diagnosis remains a challenge as HLH may initially present similarly to sepsis and many other common conditions. In these case series, all four patients initially presented with subjective fevers and extreme fatigue which had lasted one to three months. These non-specific and poorly localizing symptoms could be present in a wide variety of conditions. However, the duration of these symptoms is unusual for an acute process like bacterial sepsis in which symptoms would be more likely to progress rapidly. Persistent fevers despite an-

tibacterial therapy and negative cultures as well as cytopenias should raise suspicion for HLH. Routine testing for ferritin in patients who meet these criteria might aid in early diagnosis.

After the diagnosis of HLH is made, providers have to decide if to target the underlying cause of the inflammatory response or to suppress the hyper-inflammatory response. Given that > 60% of infection-associated HLH (IA-HLH) cases are usually secondary to EBV, it might be safe to assume EBV is the cause of a patient's HLH in the absence of malignancy, autoimmune disease or any other obvious infection (23). The appropriate course of action based on the case reports reviewed is varied. As an example, some instances of EBV-HLH improved with addition of rituximab (to HLH-2004 protocol) suggesting targeting underlying EBV infection was therapeutically advantageous. However, in some instances the patients became sicker with worsening cytopenias following chemotherapy initiation (23). Patient 1 as reported here initially showed good response to HLH-2004 chemotherapy protocol. However, as disease relapsed and patient's health became more tenuous, the risks of further cycles of chemotherapy seemed to outweigh the benefits. Patient 3's poor clinical status at presentation and the rapidly progressing deterioration of the health of patient 4 made initiation of relatively toxic chemotherapy clinically inappropriate. Unfortunately, the outcome of these patients was fatal regardless of the treatment course chosen.

Future directions should include exploring the various mechanisms by which HLH is stimulated so as to aid in the development of more effective strategies. As shown in a recent publication, monoclonal antibodies to CD47 (anti-hCD47 mAb) in vivo led to phagocytic elimination of multiple tumor types and also prevented metastasis (59). Future research on chemotherapeutic targets of the CD47-SIRPα pathway might also lead to discovery of more potent therapies for HLH.

This study adds to the body of the literature by comparing and contrasting the clinical presentation and outcomes of adult patients. The concise review of conditions associated with HLH will also be helpful as clinicians search for the underlying etiology of HLH.

In conclusion, HLH is a fatal disease that usually mimics (or is triggered by) other common conditions. Early suspicion and prompt diagnosis is crucial to improved outcomes. Therapy should be individualized considering patient's baseline health, clinical presentation, and the suspected underlying trigger for HLH.

Abbreviations

HLH, hemophagocytic lymphohistiocytosis; SIRS, systemic inflammatory response syndrome; BM, bone marrow; FHL, familial hemophagocytic lymphohistiocytosis, EBV, Epstein Barr virus; CMV, cytomegalovirus; IVIG, intravenous immunoglobulin; SCT, stem cell transplantation; CTL, Cytotoxic T lymphocytes; NK, natural killer cells.

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A very unusual case of food allergy, between FPIES and IgE-mediated food allergy

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Key words

Diagnosis; egg; food protein induced enterocolitis syndrome; IgE-mediated food allergy

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Summary

Food protein induced enterocolitis syndrome (FPIES) is a food-related gastrointestinal hypersensitivity disorder, probably non-IgE-mediated. Over the years, various diagnostic criteria have been proposed to identify FPIES. In the last few years, there was an increased interest from researchers about FPIES's syndrome, that frequently brought to discover new aspects of this disease. We describe an unusual case of FPIES to egg in a 21-months-old child, because of its clinical characteristics that reflect some aspects of IgE-mediated allergy and other of non IgE-mediated allergy. Although we believe that the most correct diagnosis for our case is FPIES, we think also that this is undoubtedly an atypical form. This is in fact, the first description of a patient who simultaneously has both clinical expressions of IgE-mediated FA that of FPIES. Our case highlights the need to review criteria for FPIES diagnosis.

Introduction

Food protein induced enterocolitis syndrome (FPIES) is a food-related gastrointestinal hypersensitivity disorder, probably non-IgE-mediated. Symptoms of the acute form include projectile and repetitive vomiting, diarrhea, lethargy, and, in more severe cases, also dehydration, hypotension, and shock. Symptoms usually occur between 1-4 hours from ingestion of the guilty food (1). Over the years, they have been proposed several diagnostic criteria to identify FPIES (1-5). There are some differences among these, probably due to the lack of established validations of proposed criteria. Moreover, interest from researchers brought to discover new aspects of this disease. For example, recently a case of FPIES to mushrooms was published (6), it was unusual because the first episode occurred in a 7-year-old girl. The authors highlight the need to revise the current diagnostic criteria of FPIES which currently provide that the age of the first episode is no more than two years.

We describe a case of FPIES to egg, also unusual because of its clinical characteristics that reflect some aspects of IgE-mediated allergy and others of non IgE-mediated allergy. Also, our case highlights the need to review criteria for FPIES diagnosis. In particular, we consider appropriate to delete the criterion "absence of symptoms that may suggest an IgE-mediated reaction".

Case report

A 21-months-old child was conducted to our ambulatory for evaluation of a suspected egg allergy. At the age of 10 months, he ate a teaspoon of raw egg mixed with hot soup and soon after he went to sleep. About two hours later, his grandmother heard him complain and she noticed some wheals of urticaria on the face of the baby that increased in few minutes. The child presented also a single vomiting and appeared moderately lethargic and pale. One month before, he had eaten twice the yolk of

a boiled (for 10 minutes) egg without adverse reactions. Since then, the child has no longer eaten egg.

We performed skin prick test (SPT) and results were the following: raw egg (mixed albumen and yolk) = 6 mm (mean wheal diameter), boiled egg albumen = negative, boiled egg yolk = negative, baked egg (muffin) = negative, commercial extract of egg albumen (Lofarma, Italy) = 3 mm (mean wheal diameter), commercial extract of egg yolk (Lofarma, Italy) = negative. After a week, the child performed an OFC with baked egg (muffin). He ate 50 grams of muffins (containing 1.5 grams of egg protein) and three hours later showed repeated and projectile vomiting, mild pallor and lethargy. Symptoms resolved spontaneously within 2 hours. The same day, before the OFC, a rub test with raw egg was performed on the skin of the face and back of the child and, after 20 minutes from the beginning of the test, small wheals of urticaria, rash and itching appeared and increased progressively in about 10 minutes.

Four weeks later, because of the positive result of SPT and rub test with raw egg, the child performed an OFC with raw egg to establish the possibility of an IgE-mediated allergy to this food. SPT with raw egg performed the same day of the OFC resulted again positive (mean wheal diameter = 5 mm), while rub test with raw egg was negative. Patient gradually assumed a half of a raw egg, according to the methodology of the OFC for IgE-mediated FA, as suggested (7). Two hours after the ingestion of the first dose (and 20 minutes after the ingestion of the last dose) he presented a vomit without pallor and without lethargy. 0.2 mg/kg of ondansetron were administered intramuscularly. Nevertheless, the child presented other episodes of vomiting (overall 5 episodes), associated to pallor and lethargy. Blood pressure was always normal. After 4 hours from the beginning of symptoms, the child was fine.

Discussion

We think that this case is an unusual clinical expression of a single FA based on mixed mechanism, IgE and non-IgE-mediated. The characteristics that suggest an IgE-mediated FA are: a) the urticaria after the ingestion of raw egg at the age of 10 months; b) the positivity of SPT with raw egg; c) the positivity of the first rub test. Instead, the following are characteristics that suggest a non-IgE-mediated mechanism: a) onset of symptoms 2-3 hours later the ingestion of half-hard egg at the age of 10 months and during the OFC with muffin; b) the failure of muffin OFC (the egg thus processed is usually tolerated by those patients who have an IgE-mediated egg allergy); c) the absence of urticaria during the OFC with raw egg; d) the negativity of the second rub test with raw egg. Of course, it is strange that the rub test was negative the second time and we do not know how to explain it. We believe a false negative possible (the second time), for example due to an inaccurate execution, while we consider a false positive unlikely (the first time). We do not have a sIgE profile at molecular level in our patient. Some authors (8-11) have measured the sieric ovomucoid specific IgE, in order to identify a cut-off value with positive predictive value > 95% in predicting adverse reaction during OFC with baked egg, reporting very variable values (from 3.3 kU/L to 50 kU/L).

Although we believe that the most correct diagnosis for our case is FPIES, we also think that this is undoubtedly a very atypical form. Cases of FPIES shifted to an IgE-mediated FA (12) and cases of IgE-mediated FA shifted to FPIES have already been described (13), but to our knowledge this is the first description of a patient who simultaneously has both IgE-mediated FA and FPIES clinical expressions. Like that of Serafini et al (6), also our case highlights the need to review criteria for FPIES diagnosis. In particular, we consider appropriate to delete the criterion "absence of symptoms that may suggest an IgE-mediated reaction".

The OFC with raw egg raised some doubts in us. We have gradually performed it, according to the methodology of IgE-mediated allergy, as suggested by guidelines (7). On that occasion, the child began to vomit two hours later the ingestion of the first dose and 20 minutes later the ingestion of the last dose. What will have been the dose responsible for symptoms? If the first dose was responsible, the reaction was compatible with FPIES, while if the last dose was responsible, the reaction was compatible with an IgE-mediated FA.

Finally, our case represents the first description of a therapeutic failure of the intramuscular ondansetron in controlling vomiting during OFC performed for FPIES.

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Anaphylaxis to hidden potato allergens in a peach and egg allergic boy

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Key words
Food anaphylaxis; clinical aspects; hidden allergens; component resolved diagnosis

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Summary
More than 170 foods have been identified as being potentially allergenic. However, a minority of these foods cause the majority of reactions. Sweets are frequently implicated in allergic reactions in children with cow's milk, egg, nuts or fruits allergy, and they are the most relevant foods investigated as responsible allergens.
We report an anaphylactic reaction to candies in an egg and peach allergic boy. We performed a study to identify responsible allergens for the reaction. We investigated hidden egg and peach allergens in the candies, but they were not found. Finally, the causative allergen resulted to be a vegetable protein from potato peel. We diagnosed a new allergy in our patient and Sol t 4 was identified as the responsible allergen of the anaphylactic reaction.
We conclude that responsible allergens should always be studied and identified in whatever allergic reaction in order to prevent new reactions.

Introduction

Sweets are frequently implicated in children's allergic reactions with cow's milk, egg, nuts or fruits allergy, and those are the most relevant foods that have been investigated as the responsible allergens.
We reported an anaphylactic reaction to candies in an egg and peach allergic boy. A study was performed to identify potential allergens present in candies and their origins. The causative allergen was identified as a vegetable protein, used as a thickener ingredient in the manufacture of one of the candies. It was a protein not related to any previously diagnosed allergy in our patient. Finally, we identified the Sol t 4, a peel potato protein, as the responsible allergen of the anaphylactic reaction.
We conclude that, in whatever allergic reaction, the responsible allergens should always be studied and identified in order to prevent new reactions.

Case report

A 21-month-old boy developed, ten minutes after ingesting some candies (with trade names "Lolipop", "Fresa besito" and "Nube fresa"), perioral urticaria with lip edema, abdominal pain, vomiting and generalized urticaria. The reaction subsided in 3 hours with H1 antihistamine and corticosteroids treatment. At that time, he was tolerating cow's milk, boiled egg, meat, fish, cereals and vegetables including boiled potato, legumes (lentils, soy, beans, chickpeas, peanuts) and other fruits including kiwi. We discharged as cofactors exercise, infections or drugs. Some days later, he developed pruritus and perioral hives immediately after ingesting soya, green peas or lentils. Previously, at twelve months, we had studied the patient because of immediate generalized urticaria after ingesting egg and perioral urticaria coinciding with peach ingestion, and the baby was diagnosed with egg and peach allergy. He had mild atopic dermatitis but he had

not had bronchospasm episodes, and his family had no history of allergic disease.
We performed a study to identify potential allergens present in candies and their sources.

Materials and methods

All candies implicated in the reaction were investigated. Their labels were studied to ascertain the ingredients, and the original components were supplied by the manufacturer.
Skin prick by prick tests (SPPT) with an aqueous solution of each candy were performed on the patient and on 10 control children (5 with egg and 5 with peach allergy) to investigate hidden egg or peach allergens. Then we completed SPPT on the patient with natural components of candies which resulted positives (peel, and raw and boiled pulp potato).
Skin prick tests (SPT) were performed with a panel of food allergens including cow's milk and white egg proteins, peach, (rPru p 3) and (rPru p 4), potato, legumes and nuts, using commercial extracts and histamine and saline solution as positive and negative controls (ALK-Abelló laboratories, Madrid, Spain); and with aqueous extract of each one of the ingredients (10 mg/ml) of the candies showing a positive SPPT response. Skin tests were considered positive if average diameter was equal or greater than histamine diameter. Total and specific IgE were assessed by ImmunoCAP and microarrays (ISAC IgE) (Thermo Fisher, Uppsala, Sweden).
IgE-immunoblot and immunoblot inhibition experiments were carried out to investigate the responsible allergens. Samples (10 mg of peel potato extract and 2 mg of Pru p 3) were separated by SDS-PAGE and replica gels were electro-transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking, the membranes were incubated overnight with patient's serum (1:3 dilution), and with polyclonal rabbit antibodies produced against Pru p 3 (peach LTP; dilution 1:1000). Detection of IgE-binding components was achieved by means of enhanced chemiluminescence, according to the manufacturer's instructions (Amersham Biosciences, Little Chalfont, UK). The inhibition assays were performed incubating Pru p 3 (5 µg/mL) with patient's serum 3 h at room temperature, previously to immunoblot.
The identification of the peptide was performed by peptide-mass fingerprinting.

Results

Information on the labels of the candies ("Lolipop", "Fresa besito" and "Nube fresa") ingested by the patient within one hour before the reaction, did not include egg or peach ingredients. The patient showed positive SPPT to "Lolipop" (7 mm mean diameter) and negative to "Fresa besito" and "Nube fresa". The control subjects showed negative SPPT to the three candies.

"Lolipop" label declared as ingredients: sugar, glucose syrup, vegetable fat, maltodextrin, water, aromas, vegetable protein, starch, soy lecithin, E-330 (citric acid) and E-120 (cochineal red). The SPT to each of these components (10 mg/ml) resulted positive only to the vegetable protein (mean diameter 11 mm). The source of vegetable protein was identified by the manufacturer as potato peel proteins. Results of skin tests, food specific IgE and tolerance are shown on the **table**.
The patient's serum recognized Pru p3 and a low molecular weight IgE-binding band in the potato peel extract. To identify the nature of this band, inhibition assays were conducted. Pru p 3 was capable of self-inhibition but did not inhibit the IgE-binding reactivity of the potato peel band. The IgE-binding potato band wasn't recognized by polyclonal rabbit antibodies against Pru p 3 (**figure 1**). Finally this potato allergen was identified as Sol t 4, a protease inhibitor belonging to the family of Kunitz-type soybean trypsin inhibitors.
When the study was concluded, the patient was tolerating cooked potatoes, and symptoms with legumes (soya, green peas, lentils and chickpeas) had disappeared. However, he was on a kiwi, walnut and peanut free diet. The patient's mother didn't accept a challenge to prove tolerance of patient to these foods.

Figure 1 *- Results of IgE-immunoblot and immunoblot inhibition experiments:*
a) Line 1 vegetable protein of "Lolipop" (peel potato) and line 2 Pru p 3 separated by SDS–PAGE and stained with Coomassie.
b) Replicas of lines 1 and 2 immunodetected with patient'serum (Immunoblot; dilution 1:3.
c) Replicas of lines 1 and 2 immunodetected with patient'serum preincubated with Pru p 3.
d) Replicas of lines 1 and 2 immunodetected with anti LPT antibodies.

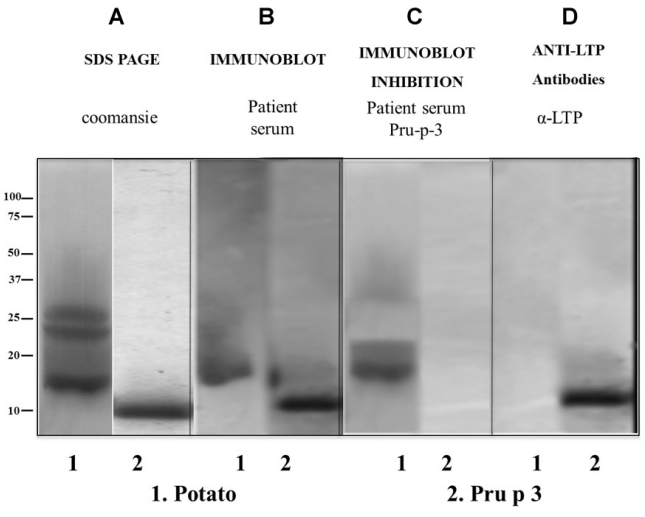

Table 1 *- Results of allergic study and tolerance to different foods and latex. Results of skin test (prick and prick by prick) and specific IgE (Cap and Microarrys) and tolerance at time of the study. Tolerance: Yes, No or NI (not introduced in the patient diet).*

	Prick by Prick	Prick	Specific IgE		Tolerance
	Mean diameter (mm)		Cap U/L	Microarrays ISU	
"Lolipop" candy	7				
"Fresa besito" candy	0				
"Nube fresa" candy	0				
White egg		5.3	0.49	0.0	Yes (boiled egg)
Ovalbumin		6	0.5	0.0	
Ovomucoid		0	0.01		Yes
Cow's milk		0	0.01		NI
Sesame		0	0.02		NI
Mustard		0	0.01		NI
Peanut		0	0.12		NI
Walnut		0	0.04		Yes
Latex		0	0.01		
Peel potato protein		11			No
Potato			6.12		
Peel	7				
Boiled pulp	5				Yes
Raw pulp	10				
Tomato			0.62		Yes
peel	10	6			
pulp	0				
Peach		8	13.2		No
Pru p 4		0	0.01		
Pru p 1			0.00	2.9	
LTP rPru p 3		6.5	15.10		
nsLTP rAra h 9			0.5	0.5	
rCor a 8			0.0	0-01	
nJug r 3			1.1	1.1	
nArt v 3			0.0		
nOle e 7			0.0		
rPla a 3			0.3		
Peas		3	0.52		No
Lentil		4	0.65		No
Soya		3	0.40		No
rGly m 4			0.00	0.0	
nGly m 5			0.07	0.0	
nGly m6			0.04		
Kiwi		8	5.53		Yes
n Act d1				2.7	
n Act d 2				0.3	
nAct d 5				0.0	
r Act d 8				0.0	
D pteronyssinus					
Der p 1		0		0.0	
Der f 1		0		0.0	

Discussion

Food allergens in food supplements and sweets have been implicated as elicitors of anaphylactic reactions in allergic children, and hidden components are sometimes identified as causal allergens (1,2). Our patient developed an anaphylactic reaction immediately after eating candies. He was allergic to egg and peach, and showed a positive SPPT to "Lolipop" candy. Although these components were not declared in the candy labels, they could be hidden allergens in some sweets. However, the negative results of SPPT with "Lolipop" on egg or peach allergic control subjects ruled out this possibility. Finally, the vegetal protein component in "Lolipop" (protein from peel potato) was confirmed as the responsible allergen. The proved tolerance to cooked peeled potato by our patient and his intense sensitization to Pru p3, made us think about the possibility of a lipid transfer protein (LTP) from potato as the responsible allergen. However, IgE in the patient's serum recognized a band of approximately 15 kDa in the vegetable protein, which were neither inhibited by Pru p 3 or recognized by specific polyclonal antibodies against plant LTP. The allergen detected by the patient's serum was identified as a cysteine protease inhibitor belonging to the family of soybean trypsin inhibitors Kunitz type (Sol t 4).

Potatoes represent an important part of the worldwide diet. Allergic reactions to this foodstuff are uncommon, and usually result from ingestion, mainly in children. Castell et al. (3) reported anaphylaxis to white potato in a girl, and they demonstrated specific IgE antibodies directed against several potato proteins ranging from 14,000 to 40,000 KDa. Allergic reactions to contact with raw potato has been reported more frequently in adults, usually in the form of an oral contact dermatitis or contact urticaria (4,5), but asthma, rhinoconjunctivitis, wheezing or even anaphylaxis had also been described (6,7).

Potato contains a number of allergens, ranging from 16 to 65 kDa in size, of which a few have been characterized. Smith et al. (8) demonstrated that 75% of potato-sensitised subjects reacted to Sol t 1 (patatin) a 43 kDa allergen. Sol t 2, Sol t 3 and Sol t 4 have molecular masses ranging from 16 to 20 kDa and have been identified as cathepsin D-, cysteine-, and aspartic protease-inhibitors belonging to the family of Soybean trypsin inhibitors (Kunitz type); Seppala et al. (9) showed IgE binding to Sol t 4 in 67%, Sol t 2 in 51%, and to Sol t 3 in 43% of the sera of atopic children.

A study in children up to 4 year old with suspected food allergy showed that 70% of children had positive SPT to potato and IgE antibodies to a Kunitz-type soy trypsin inhibitor (KSTI) and the 75% of children with suspected soya allergy, had IgE antibodies to Sol t 2-4. A marked allergenic cross-reactivity was demonstrated between Sol t 2-4 and these KSTI allergens. The study concluded that in children with positive SPT and serum IgE to soy, there may be cross-reactive IgE antibodies to potato allergens and vice versa (10). Our patient developed oral allergy transient symptoms to legumes and we demonstrated low levels of specific IgE to them.

On the other hand, although previously the patient had tolerated kiwi, the study showed an intense sensitization particularly to nAct d 1, a cysteine protease. After the reported reaction, we could not verify that the child tolerates this fruit because his mother didn't approve a controlled challenge.

We conclude that in food allergic reactions the causative allergens should be thoroughly investigated, even in patients with a previous diagnosis of allergy to common foodstuffs.

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