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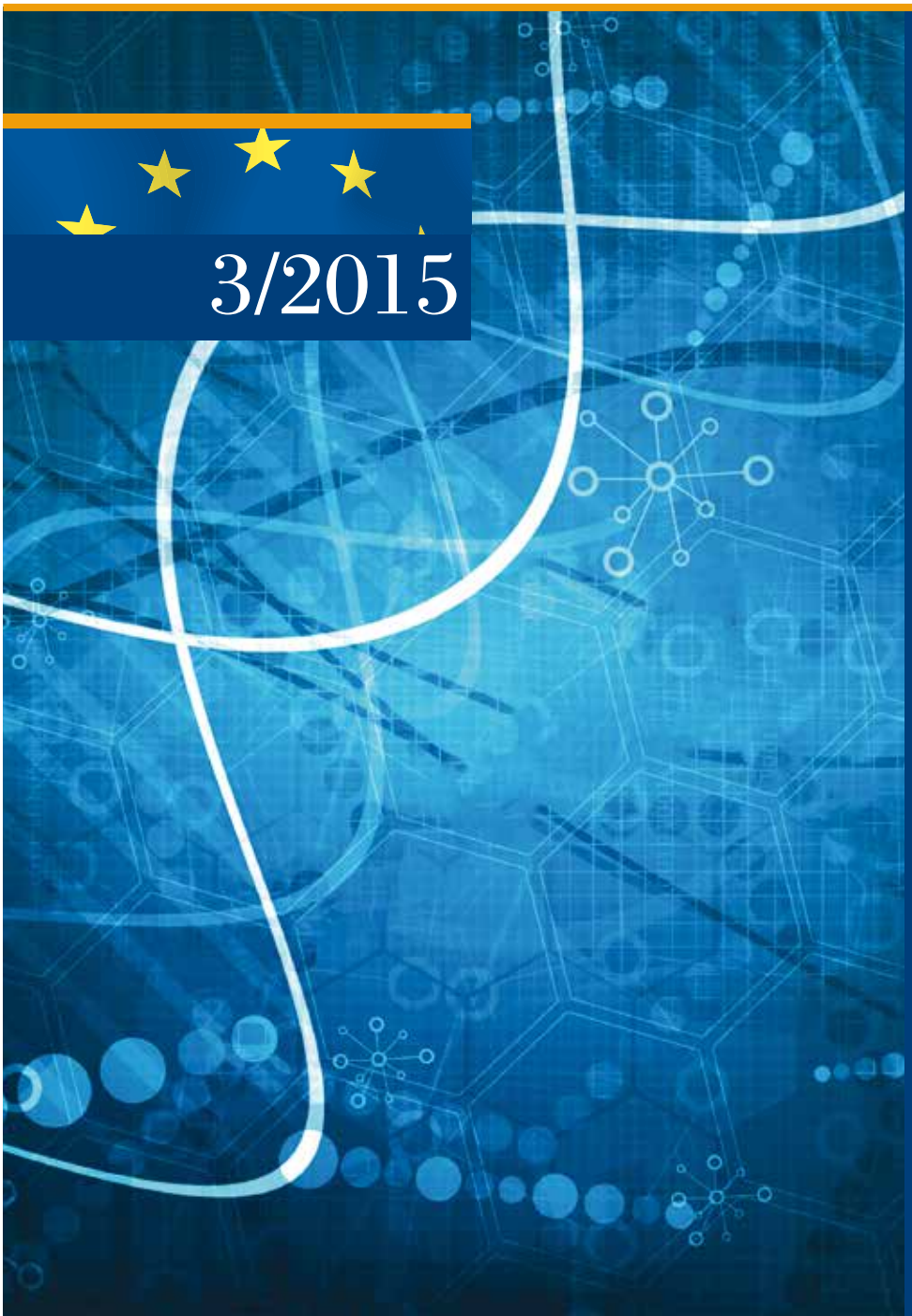

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1. **Summary and key words**: english, limited to 15 lines.
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3. **Materials and methods**.
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The oral food desensitization in the Italian allergy centres

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*Italian Society of Pediatric Allergy and Immunology (SIAIP) - Food Allergies and Specific Immunotherapy Committees (2010-2012)

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Key words

Food allergy; oral food desensitization; oral immunotherapy; specific oral tolerance induction; sublingual oral food desensitization; food tolerance; immunotherapy safety; cow's milk allergy; hen's egg allergy; egg allergy

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Summary

Background. *Attempts aimed at inducing food tolerance through oral food desensitization (OFD) for the treatment of IgE-mediated food allergies are increasing. In Italy, a number of allergy centres offer this procedure.* ***Objective.*** *To collect information on how these centres are organized, how patients are selected, the methods used to administer OFD and how adverse reactions are managed.* ***Methods.*** *A questionnaire was e-mailed to all the Italian allergy centres offering OFD.* ***Results.*** *The survey shows a high degree of variability between centres. A correct diagnosis of food allergy is crucial for selecting patients for OFD. In the Italian allergy centres, oral food challenges are mostly open label (84%), but in 16% of cases they are single-blind (8%) or double-blind (8%). A high proportion of allergy centres (83%) offer OFD to children presenting forms of anaphylaxis triggered by traces - or very low doses - of food allergen. The majority of allergy centres (76%) enroll patients over 3 years of age, with 44% enrolling patients above the age of 5. Not-controlled asthma, unreliability of parents in the management of OFD and/or risk of adverse events, are the main reasons for exclusion from the procedure.* ***Conclusion.*** *Although OFD may sometimes be successful and may be considered a valid alternative to an elimination diet, further randomized controlled trials are needed, in order to clarify some controversial points, such as the characteristics of the child undergoing OFD, and the methods of food preparation and administration. Moreover, further studies should further investigate OFD safety, efficacy and costs.*

Introduction

Food allergy (FA) is a common condition, especially in children (1). Besides recommending avoiding the offending food (2), the induction of food tolerance through oral food desensitization (OFD) is proposed today for the treatment of the IgE-mediated forms of this condition (3-12). OFD is achieved through the administration of incremental doses of the offending food, which are progressively increased up to a predetermined top dose, or to the maximum tolerated dose. This method aims at inducing desensitization and, possibly, tolerance to the offending food.

There are numerous case reports and randomized controlled trials (RCTs) investigating OFD (3-12), but they are difficult to compare as they are not homogeneous in terms of the enrolled population, the offending food, its administration route, the dosage and the setting (home, hospital, day-hospital, outpatient clinic).
Up to May 2011, Brozek et al. (13) found that only 5 RCTs met the pre-established inclusion criteria. The RCTs analyzed (involving 218 patients) showed that OFD, compared to the elimination diet, increased the likelihood of achieving oral tolerance to cow's milk (CM). OFD adverse events included frequent local reactions, mild laryngospasm and mild asthma. Results obtained from observational studies were consistent with those obtained from RCTs. The safety of OFD represents a pivotal issue in patients treated with this active treatment for FA. Indeed, between 10% and 36% of patients could not complete the protocol due to adverse reactions (3-11,14). Although OFD may induce a variable level of desensitization, it remains unclear whether this therapeutic approach results in complete, long-lasting tolerance (15).
As the practice of OFD is quite common in Italy, we administered a questionnaire in the structures where OFD is practised, with the aim of taking a snapshot of the procedures concerning OFD adopted in Italy, in order to give targeted guidance on the standardization of this therapeutic procedure.

Materials and methods

This survey (conducted between April and November 2012) was conducted in the Italian allergy centres offering OFD, which are registered with the Italian Society of Pediatric Allergy and Immunology (SIAIP). In addition, an e-mail was sent to the main Italian pediatric allergy forums. A total of 55 allergy centres were thus identified and a questionnaire containing 26 multiple choice questions was e-mailed to them. The questions were divided into the following sections:

a. Type of services and availability of an anesthetist (questions 1-2, **table 1**)
b. Patient selection criteria (questions 3-7, **table 2**)
c. Methods of OFD execution (questions 8-15, **table 3**)
d. Management of adverse reactions occurring during OFD (questions 16-22, **table 4**)
e. Follow-up management (questions 23-26, **table 5**)

The data obtained were analyzed through descriptive statistical analysis.

Results

Type of service and availability of an anesthetist in the allergy centre (**table 1**). Twenty-four out of 55 allergy centres completed the questionnaire; 50% of them had treated 1-20 children and 37,5% between 21-100 children. Only 12.5% had treated more than 100 children. All the centres had an anesthetist on call in case of severe adverse reactions.
Patient selection criteria (**table 2**). While 75% of centres used OFD only for children with an IgE-mediated FA, a further 25% used it also for non-IgE-mediated conditions. As for the severity of symptoms, 68% considered the main indications for OFD to be anaphylaxis (even caused by traces of food), and children with partial tolerance. A lower proportion of allergists (32%) considered OFD only in children with severe conditions.
In terms of the diagnosis of FA, oral food challenge was open-label in 39% of cases, single-blind in 9% and double-blind in 9%. In 27.8% of the centres the diagnosis was based on clinical history and on the positivity of the skin prick test (SPT)/specific IgEs for the offending food. The age threshold for OFD was 6 years in 11.5% of the centres and 5 years in 38%. OFD was administered to children older than 3-4 years in 15.4% of the centres, and only in 8% of centres it was administered to children between 1-2 years old. The unreliability of parents (38.2%) and non-controlled asthma (32.7%) were the main criteria for exclusion from OFD. Anaphylaxis caused by the offending food resulted in exclusion from OFD in 12.7% of the allergy centres, and 9.1% of them also excluded patients living far from an emergency unit.
Methods of OFD execution (**table 3**). As regards the type of protocol adopted, 69.2% of the allergy centres used a slow protocol (over 2-6 months), while 15.4% used a rush protocol (lasting a

Table 1 *- Type of services and anesthetist availability.*

		Answer A	Answer B	Answer C	Answer D	Answer E	Answer F	Answer G
#1	Number of children submitted to OFD in the AC	1 to 5	6 to 10	11 to 20	21 to 50	51 to 100	101 to 200	> 200
		3 (12.5%)	2 (8.3%)	7 (29.2%)	5 (20.8%)	4 (16.7%)	2 (8.3%)	1 (4.2%)
#2	Availability of an anesthetist during the procedure	Yes	Yes, on request	NO				
		0 (0%)	24 (100%)	0 (0%)				

AC = Allergologic Centre

Table 2 *- Patient selection criteria for OFD.*

		Answer A	Answer B	Answer C	Answer D	Answer E	Answer F	Answer G
#3	Type of food related condition in children undergoing OFD	IgE-mediated FA	Not-IgE mediated (FPIES) condition	A + B				
		18 (75.0%)	0 (0.0%)	6 (25.0%)				
#4	Characteristics of patient undergoing OFD	Anaphylaxis induced by traces or very low doses	Partial tolerance	(A + B)				
		4 (16.0%)	4 (16.0%)	17 (68.0%)				
#5	FA diagnosis methods	Open-label OFC (A1) Single-blind OFC (A2) Double-blind OFC (A3)	Convincing clinical history for anaphylaxis + SPTs/IgEs positivity	Convincing clinical history in the previous year (indep. from SPT/IgEs for the offending food)	Suggestive clinical history of FA (NOT of anaphylaxis) in the previous year and SPT/IgEs+ for the offending food	Late clinical history of FA with negative SPT/IgEs		
		21(A1) (39.0%) 5 (A2) (9.0%) 5 (A3) (9.0%)	15 (28.0%)	0 (0.0%)	6 (11.0%)	2 (4.0%)		
#6	Age threshold for OFD	At diagnosis (any age)	After 1st year	After 2nd year	After 3rd year	After 4th year	After 5th year	After 6th year
		1 (3.8%)	2 (7.7%)	3 (11.5%)	4 (15.4%)	4 (15.4%)	9 (34.6%)	3 (11.5%)
#7	Exclusion criteria	Anaphylaxis induced by the offending food	Not-controlled asthma	Physical activity-induced asthma	Atopic dermatitis	Concomitant not-food-dependent allergy	Unreliability of parents	Excessive distance from the ED
		7 (12.7%)	18 (32.7%)	0 (0.0%)	1 (1.8%)	3 (5.5%)	21 (38.2%)	5 (9.1%)

FA = Food Allergy
OFC = Oral Food Challenge
ED = Emergency Department

few days) and 15.4% used a mixed protocol (rush + slow). As regards the dosage, 56% of the allergy centres allowed home administration of incremental doses of the offending food, while 44% only incremented the doses in the hospital setting. The oral route was most frequently used (84%), with sublingual administration being less common (16%). OFD was administered to patients with allergy to CM (42.9%), egg (37.5%), wheat (10.7%), fish (5%), peanut (1,8%) and hazelnut (1,8%), that were mostly administered uncooked. Some allergy centres administered the food in a wheat matrix (21.4%) or as a baked food (28.6%). In terms of the initial dosage of the food, in 37.4% of the centres a dosage lower than the one provoking a reaction in the oral food challenge was used. In 7.5% of the centres, the initial dosage was based on the clinical history or on the SPT end-point (11.2%). Dosage administration was mainly carried out in day-hospital settings (46.7%) or at home after day-hospital or hospital admission (23.3%), or directly at home in the case of slow increments. However, major increments or doubled doses were administered in a day-hospital setting (20%). In a small proportion of centres, OFD was administered during hospitalization (6.7%) or in the outpatient clinic (3.3%). During home administrations, 54.8% of allergists were available on their mobile phones 24 hours per day, while 9.7% were available in specific time slots. Nineteen percent of the allergy centres communicated with families via e-mail, compared to 9.7% of patients, who in case of need, were obliged to refer to the emergency department of the structure responsible for their OFD administration.

Management of adverse reactions in the course of OFD (**table 4**). The main criterion for the interruption of OFD was the occurrence of anaphylaxis triggered by the administration of low doses (24.7%) followed by non-controlled asthma (22.4%).

Table 3 *- Methods of OFD execution.*

		Answer A	Answer B	Answer C	Answer D	Answer E	Answer F	Answer G
#8	Type of protocol	Rush (rapid. in a few days)	Slow (2 to 6 months or more)	Mix (Rush + Slow)				
		4 (15.4%)	18 (69.2%)	4 (15.4%)				
#9	OFD increment method	Dose increase in hospital setting (not at home)	Dose increase in hospital setting and at home					
		11 (44.0%)	14 (56.0%)					
#10	Administration routes	Sublingual (without swallowing)	Sublingual (with subsequent swallowing)	Epicutaneous	Oral			
		2 (8.0%)	2 (8.0%)	0 (0.0%)	21 (84.0%)			
#11	Food	Cow's milk	Egg	Wheat	Fish	Peach	Peanut	Hazelnut
		24 (42.9%)	21 (37.5%)	6 (10.7%)	3 (5.4%)	0 (0.0%)	1 (1.8%)	1 (1.8%)
#12	Food preparation	Raw	Cooked	In wheat matrix	Freeze-dried			
		21 (47.6%)	12 (28.6%)	9 (21.4%)	1 (2.4%)			
#13	Initial dose criteria	Very low predetermined dose (decreasable if clinical history of anaphylaxis with low doses)	Based on the SPT wheal diameter	Based on the SPT end points	Based on the specific IgEs level	Lower than the one provoking a reaction in the OFC	Lower than the one based on the clinical history	
		10 (37.4%)	0 (0.0%)	3 (11.2%)	0 (0.0%)	10 (E1) (37.4%) 1.75 (E2) (6.5%)	2 (7.5%)	
#14	Setting	Day-Hospital regimen	Hospital admission regimen	At home after Day- Hospital and/or Hospital admission	At home for slow increments and in Day-Hospital for major increments or doubled doses	Outpatient clinic		
		14 (46.7%)	2 (6.7%)	7 (23.3%)	7 (20.0%)	1 (3.3%)		
#15	At-home patient management and communication with the AC	Parents can call the doctor on mobile 24 hours a day	Parents can call the doctor on mobile in specific time slots	Parents can only refer to the AC during opening hours	Only email communications	Parents can bring the patient to the ED (where data of all children undergoing OFD are available)	Other	
		17 (54.8%)	2 (6.5%)	1 (3.2%)	6 (19.4%)	3 (9.7%)	2 (6.5%)	

AC = Allergologic Centre OFC = Oral Food Challenge ED = Emergency Department

Twenty percent of the allergy centres considered the parents' ability to manage possible adverse events to be crucial.

If the child presents mild to moderate reactions during OFD, 53.8% of the allergy centres continue the procedure with lower doses of the food, which are later incremented if no adverse symptoms occur. In 15.4% of the allergy centres the trigger dose is administered until the symptoms disappear. Some of the allergy centres administer antihistamines (11.5%) or interrupt the protocol, but in such cases the maximum tolerated dose is maintained (11.5%). To the contrary, some other centres step back and prescribe allergen avoidance (3.8%).

Table 4 *- Management of adverse reactions occurring during OFD.*

		Answer A	Answer B	Answer C	Answer D	Answer E	Answer F	Answer G
#16	OFD interruption criteria after adverse reactions	Non-controlled asthma following OFD administration	Systemic anaphylaxis following very low doses	After 12 months, impossibility to achieve a minimum dose able to protect from reactions occurring after consumption of food traces	Inability of parents to manage adverse events	Repeated ED admissions		
		19 (22.4%)	21 (24.7%)	16 (18.8%)	17 (20.0%)	12 (14.1%)		
#17	OFD management in case of mild to moderate reactions	The trigger dose is re-administered without increment, and is increased after symptoms disappear	The dose is decreased of some steps and is increased when symptoms disappear	The dose is administered with wheat matrix	The patient is pre-treated with antihistamines for few days	The protocol is interrupted and the maximum tolerated dose is maintained	The protocol is interrupted and the children is prescribed an elimination diet	
		4 (15.4%)	14 (53.8%)	1 (3.8%)	3 (11.5%)	3 (11.5%)	1 (3.8%)	
#18	OFD management in case of moderate to severe reactions and/or anaphylaxis	The trigger dose is re- administered without increment, and it is increased after symptoms disappear	The dose is reduced of some steps and is increased when symptoms disappear	The dose is reduced of some steps; the patient is pre-treated with antihistamines and when the symptoms disappear the dose is increased more slowly	The protocol is interrupted and the maximum tolerated dose is maintained	The protocol is interrupted and the children is prescribed an elimination diet for the offending food		
		1 (3.8%)	12 (46.2%)	4 (15.4%)	4 (15.4%)	5 (19.2%)		
#19	Drugs used in case of adverse reactions	Nebulized epinephrine (A1) Epinephrine IM (A2)	Nebulized Cortisone (B1) Cortisone IM (B2) Cortisone IV (B3) Cortisone OS (B4)	Antihistamine IM (C1) Antihistamine IV (C2) Antihistamine OS (C3)	inhaled β2-agonists			
		10 (A1) (8.1%) 23 (A2) (18.5%)	4 (B1) (3.2%) 8 (B2) (6.5%) 13 (B3) (10.5%) 13 (B4) (10.5%)	7 (C1) (5.6%) 8 (C2) (6.5%) 19 (C3) (15.3%)	19 (15.3%)			
#20	Antihistamine administration during OFD	Only in case of adverse reactions (possibly associated with other drugs)	During the whole protocol, independently from the subject	During the whole protocol, only in high-risk subjects				
		18 (78.3%)	1 (4.3%)	4 (17.4%)				
#21	Antihistamine molecule	Cetirizine	Oxatomide	Chlorpheniramine	Other			
		20 (87.0%)	1 (4.3%)	1 (4.3%)	1 (4.3%)			
#22	Extra-dietary factors affecting OFD	Physical activity	Respiratory tract infections	Gastroenteric infections	Fasting	Drugs	Pollinic season	Other
		20 (24.4%)	18 (22.0%)	18 (22.0%)	4 (4.9%)	10 (12.2%)	11 (13.4%)	1 (1.2%)

ED = Emergency Department

Table 5 *- Follow-up management.*

		Answer A	Answer B	Answer C	Answer D	Answer E	Answer F
#23	Management after achievement of the maximum dose	Daily consumption of the maximum tolerated dose	Daily consumption of the food (not necessarily the amount achieved through the protocol)	Occasional consumption allowed (never beyond 2 days)	Occasional consumption allowed (never beyond 4 days)	Occasional consumption allowed (never beyond 7 days)	Free consumption allowed
		5 (18.5%)	8 (29.6%)	5 (18.5%)	4 (14.8%)	0 (0.0%)	5 (18.5%)
#24	Management after achievement of a partial tolerance	Daily consumption of the maximum tolerated dose	Daily consumption of the food (not necessarily the amount achieved through the protocol)	Occasional consumption allowed (never beyond 2 days)	Occasional consumption allowed (never beyond 4 days)	Occasional consumption allowed (never beyond 7 days)	Free consumption allowed
		5 (18.5%)	8 (29.6%)	5 (18.5%)	4 (14.8%)	0 (0.0%)	5 (18.5%)
#25	Adverse reactions following an occasional consumption of the food	Reactions that had occurred previously, equally or less severe	Reactions that had occurred previously, but more severe (including anaphylaxis)	Reactions different from those that occurred previously	No reactions		
		4 (36.4%)	0 (0.0%)	0 (0.0%)	7 (63.6%)		
#26	OFD immunological evaluation	Yes, at the completion on the protocol	Yes, at the completion on the protocol and every 6 months	Yes, at the completion on the protocol and annually	Yes, at the completion on the protocol and periodically at predetermined time intervals	No, never	
		2 (8.3%)	4 (16.7%)	5 (20.8%)	9 (37.5%)	4 (17.7%)	

In the case of severe reactions or anaphylaxis during OFD, lower doses were administered in 46.2% of cases, and were subsequently increased if the patient did not experience any symptoms. Some of the allergy centres pre-treated the patient with an antihistaminic drug and then slowly increased the doses (15.4%), whereas some others preferred to prescribe allergen avoidance (19.2%).

Treatment of adverse events was usually appropriate to the type of reaction.

Physical activity, respiratory tract infections and gastroenteric infections (24.4%, 22% and 22% respectively) were considered the main factors that could facilitate adverse reactions during OFD.

Follow-up management (**table 5**). If the top dose of the protocol was tolerated, 48% of the allergy centres prescribed daily consumption of the food, against 18.5%, which prescribed a free diet. When the acquired tolerance was partial, daily consumption of the food was the most common prescription (69.2%).

Children who had ingested the food occasionally during the follow-up did not have adverse reactions (63.6%) or reported reactions that had already occurred previously (36.4%). No anaphylaxis was reported.

Discussion

Type of services and availability of an anesthetist in the AC (**table 1**). All the centres rely on an anesthetist who does not participate to the procedure but is ready to intervene on request. This is important for the safe execution of OFD. In patients with a high risk of severe adverse reactions, an anesthesiologic visit before the procedure is considered useful.

Patient selection criteria (**table 2**). The majority of the centres offer OFD only to patients with exclusively IgE-mediated FA. Nevertheless, 25% also administer OFD to patients with not-IgE-mediated forms (FPIES, allergic enteropathy, eosinophilic forms). According to current evidence, even if OFD in the IgE mediated food allergy is still considered an experimental approach, there are no recommendations for the administration of OFD to patients with not-IgE mediated forms (16) and it should be done exclusively in the context of research protocols

with the purpose of verifying its efficacy. A high proportion of the allergy centres (83%) offer OFD to children presenting forms of anaphylaxis triggered by traces or very low doses of the food allergen. Even though this is a dangerous procedure, most allergy centres consider, according to evidence (6), that the long-term benefits of OFD are higher than the risks and disadvantages, because of the need for daily food consumption.
A correct diagnosis of FA is crucial for the selection of patients for OFD. In the Italian allergy centres OFD is mostly open label (84%) but in 12% of the centres the diagnosis is based on a suggestive clinical history of IgE-mediated FA, combined with positive SPTs and/or specific IgEs. This is reasonable when the reaction is immediate and clearly associated with food ingestion or when oral food challenge is too risky (4,17,18). Although double-blind, placebo controlled oral food challenge is still considered the gold standard in FA diagnosis, in some circumstances a single-blind or open-label oral food challenge is accepted (19). Considering the complexity of OFD the etiology should, in any case, be very accurately investigated.
The majority of the allergy centres (76%) enroll patients above the age of 3 and 44% above the age of 5. However, some of the allergy centres offer OFD even to children below the age of 2. The first approach considers the chance that food tolerance is achieved with age (13,20,21). The second takes into account the reduced quality of life of children with FA and their families, especially when the allergy is severe (22).
Not-controlled asthma, unreliability of parents for the management of OFD and/or risk of adverse events are the main reasons for exclusion from the procedure. Indeed, families have to respect the protocol accurately and take note of adverse reactions, trigger doses and circumstances that may facilitate the reactions. Moreover, families must be able to manage potential adverse events, both by adjusting food doses and by administering appropriate drugs. All these aspects underline the crucial role of families in the management of OFD.
Methods of OFD execution (**table 3**). Most of the allergy centres adopt a slow desensitization protocol (69.2%), which is sometimes associated with an early rush phase (15.4%). A smaller proportion of them adopt a rapid rush method (15.4%). Indeed, the dose increase is not always gradual and some allergy centres (44%) increase food doses only in controlled settings and the dosage achieved in hospital is then maintained in the following period, at home.
As far as the initial dose is concerned, about half of the allergy centres start with a predetermined, very low dose, decreasing it if an adverse reaction intervenes. However, some centres establish the first dose based on the SPT wheal diameter, or on the specific IgEs level. It is difficult to propose precise doses and time intervals for the progressive increment. Indeed, there are no comparative observations evaluating the different administration protocols. Gradual and proportional food administration should always be accurately respected and empirical and irregular approaches should be avoided, in order to guarantee an appropriate safety level (23).
Though the majority of the centres adopt the oral administration route, some are exploring the sublingual route. Even if the tolerance may be elicited by different food administration routes, the oral one seems to offer better results than the sublingual route, because it imitates what happens in natural food consumption and permits the use of much higher food doses (24).
In 80% of the centres, children submitted to OFD are allergic to CM and/or egg. To a smaller extent, desensitization to wheat is also practiced, while desensitization to foods such as peanut and hazelnut is still negligible, since the prevalence of these allergies in Italy is low.
Food is administered uncooked in 47.6%, and cooked in 28.6% of the centres. Food in wheat matrix is administered in 21.4% and freeze-dried food in 2.4% of the centres. Food preparation is still a controversial aspect of OFD, particularly with respect to egg. Consequently, while some protocols use raw egg (10,11), other protocols using freeze-dried egg have also been used (25). The possibility of inducing tolerance towards cooked egg proteins contained in cooked products would allow children to considerably broaden their diet, since children only occasionally consume uncooked egg. The risk connected with different cooking methods (poaching, frying, baking) still needs to be evaluated and the different cooking methods should be standardized (26).
Management of adverse reactions during OFD (**table 4**). Since the majority of the allergy centres also administer OFD to patients with previous history of anaphylaxis (**table 2**, 68%), care-givers should take into account possible severe adverse reactions, which may necessitate management with an appropriate therapy. Moreover, children undergoing OFD in hospital settings should receive venous access for the prompt administration of an intravenous therapy. Drugs should be prepared before administering the food dose, ready for use. Moreover, parents should always receive a copy of an action plan for drug interventions at home. After applying the action plan instructions, parents should keep in touch with the pediatrician (possibly via mobile phone).
Drugs used in case of OFD adverse reactions (intramuscular epinephrine and/or steroids) have to be chosen according to the severity of the reaction (27). As mild to moderate reactions are more frequent, antihistamines were among the most widely administered drugs. During the treatment at home, allergy centres often try to minimize the risk of adverse reactions through the daily administration of doses which are much smaller than those tolerated in hospital. Nevertheless, some protocols establish a gradual and constant food introduction at home too (4,11). Parents should be also alerted about the possibility that some

factors, such as physical exercise, respiratory and gastroenteric infections, and the administration of gastro-damaging drugs may induce the loss of food tolerance and trigger possible severe adverse reactions (28).

Follow-up management (**table 5**). When the protocol is completed, one problem is how to continue consuming the tolerated food, even in the case of partial tolerance. Indeed, while some subjects may reach a definitive food tolerance, others maintain tolerance to the offending food only if they consume it constantly, even if not daily (5,7). Some children who consumed the food occasionally, reported the occurrence of previously experienced reactions (36.4%). This finding is consistent with current literature (5), but is a very controversial point. The occasional consumption of the food should be allowed only if a significant decrease of specific IgEs or of the SPTs intensity is observed. In our opinion, subjects who, at the completion of the protocol, tolerate the food but show a high persistence of specific IgEs, should be prescribed daily consumption. Indeed, specific IgEs decrease and reduction of SPTs reaction intensity are the most reliable indicators of the efficacy of OFD. Furthermore, as we already pointed out, OFD is highly specific and IgEs decrease regards only the food to which the subject is allergic and for which he/she has been submitted to OFD (29).

The child who has reached food tolerance through OFD must be monitored by means of outpatient controls over time, in order to establish whether the achieved tolerance is persistent or not (30).

Conclusion

Our survey shows significant differences in the way the Italian allergy centres conduct OFD. Indeed, although this procedure often results in clinical success, and in selected patients may be considered a valid alternative to the elimination diet, further and appropriately designed RCTs are still needed before OFD can be considered a routine procedure in specialized centres. In addition to OFD safety, efficacy and costs, RCTs should be aimed at defining indications, food preparation methods and administration protocols. Moreover, in our opinion, further studies should also better investigate achievement of a definitive food tolerance, through an evaluation of specific immunological parameters during OFD.

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Proposal of a skin tests based approach for the prevention of recurrent hypersensitivity reactions to iodinated contrast media

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Summary

The purpose of the present work is to evaluate the efficacy of an approach that combines clinical history, skin tests results, and premedication, in preventing recurrent hypersensitivity reactions to iodinated contrast media (ICM). Skin Prick tests, Intradermal tests, and Patch tests were performed in 36 patients with a previous reaction to ICM. All patients underwent a second contrast enhanced radiological procedure with an alternative ICM selected on the basis of the proposed approach. After alternative ICM re-injection, only one patient presented a mild NIR. The proposed algorithm, validated in clinical settings where repeated radiological exams are needed, offers a safe and practical approach for protecting patients from recurrent hypersensitivity reactions to ICM.

Introduction

The incidence of hypersensitivity reactions to iodinated contrast media (ICM) has grown dramatically in recent years, together with the tremendous increase of ICM administration (1). At present, ICM are among the most frequently used pharmaceuticals for intravascular injection with over 75 million infusions per year worldwide (2,3).

According to the timing of onset, hypersensitivity reactions have been classified in immediate (IRs) and non-immediate reactions (NIRs). Immediate reactions occur within one hour after contrast administration; non-immediate reactions occur more than one hour after injection (4). Interestingly, at least for IRs, chemical structure, osmolarity and iodine content of the different ICM have been shown to influence the likelihood of developing a hypersensitivity reaction. For instance, high-osmolar ICM are not used any more, due to a higher risk of adverse events (4,5), while low-osmolar ICM are routinely used and regarded as relatively safe, with an overall frequency of adverse reactions that ranges from 0.7 to 3.1% (4). Low-osmolar ICM can be further distinguished into non-ionic monomers (iohexol, iopamidol, ioversol, iopromide, iomeprol, iopentol and iobitridol), ionic dimers (ioxaglate), and non-ionic dimers (iodixanol), with monomeric ICM being more frequently involved in IRs, and dimeric ICM in NIRs (6,7).

From a pathogenic perspective, ICM hypersensitivity reactions have traditionally been classified as non-allergic reactions, since (i) reaction on first exposure can occur, and (ii) contrast me-

dia-specific IgE antibodies have seldom been detected (8). However, during the last few years, several investigators have reported positive skin tests in patients with both IRs and NIRs, supporting an underlying allergic mechanism (9). In particular, it has been proposed that IRs may be elicited both by IgE-mediated mechanisms and by ICM direct induced histamine release from basophils and mast cells (9-11). On the contrary, most NIRs appear to be T-cell mediated, as suggested by the presence of dermal infiltrates of T cells in affected skin and by the proliferative T cell responses to the culprit ICM in vitro (4,12-14). Based on these immunological evidences, skin tests with diagnostic purposes have gained new consideration in recent years, and several studies addressed the role of skin prick tests (SPTs), intradermal tests (IDTs) and patch tests (PTs) in identifying hypersensitivity to ICM. In particular, the first "European multicenter skin test study" showed that up to 50% of patients with previous IRs and NIRs could be diagnosed by standardized skin tests, if evaluated within 2-6 months after the index reaction (15). However, despite these evidences, hypersensitivity to ICM still represents a major concern in clinical settings where repeated radiological examinations are required, as in case of malignant and chronic inflammatory disorders. In effect, the prognostic value of skin tests for the selection of safe alternative ICM in patients with previous adverse reactions to iodinated compounds remains poorly characterized. Moreover, the various published premedication protocols are not protective in cases of previous severe anaphylaxis, and do not completely guarantee patients against recurrent adverse reactions (16-19). In this sense, a large meta-analysis concluded that physicians should not completely rely on the efficacy of premedication alone since, in unselected patients, a large number of subjects need to be premedicated to prevent one potentially serious reaction (19).

Given these areas of uncertainty, in the present work we propose an algorithm that combines and integrates clinical history, skin tests performed according to international guidelines and premedication, for preventing recurrent hypersensitivity reactions to ICM.

Materials and methods

Patients. From our Database of Hypersensitivity reactions to ICM, we identified 36 consecutive patients who were tested within 2 to 6 months after the adverse reaction, in accordance with the timing indicated by international guidelines (15). Patients included in this study were referred between March 2010 and January 2014. Hypersensitivity reactions were classified as IRs when occurring within one hour after ICM injection, and as NIRs when occurring from one hour to 7 days after ICM administration (4). Immediate reactions were graded according to the Ring and Messmer classification: generalized cutaneous and/or mucocutaneous symptoms like pruritus, skin eruption, urticaria and angio-oedema (grade 1); mild systemic reactions including skin manifestations, abdominal symptoms, respiratory symptoms, cardiovascular symptoms (tachycardia) (grade 2); life-threatening systemic reactions including shock (grade 3); cardiac and/or respiratory arrest (grade 4) (20). Non-immediate reactions were defined as mild when no treatment was required, moderate when the patient responded readily to an appropriate treatment without hospitalization, and severe when the reaction required treatment in hospital, was life-threatening or resulted in death (4). All subjects signed written, informed consent for the investigations described. Since all tests were performed for diagnostic purposes, an ethical committee approval was not required for this observational analysis.

Skin testing. All patients were evaluated within 2 to 6 months after the adverse reaction and were tested with a panel of at least 3 ICM used at the Radiology Department of our Institutes in addition to the culprit agent, when known. The panel of ICM included: iohexol (Omnipaqueâ 300 mg I/mL), iomeprol (Iomeronâ 400 mg I/mL), iopromide (Ultravistâ 370 mg I/mL), iodixanol (Visipaqueâ 270 mg I/mL), iobitridol (Xenetixâ 300 mg I/mL). Skin prick tests with undiluted ICM, IDTs with 100-fold diluted, 10-fold diluted, and undiluted ICM, and PTs with undiluted ICM were performed, and interpreted according to the International Guidelines and the European multicenter study protocol respectively (15,21).

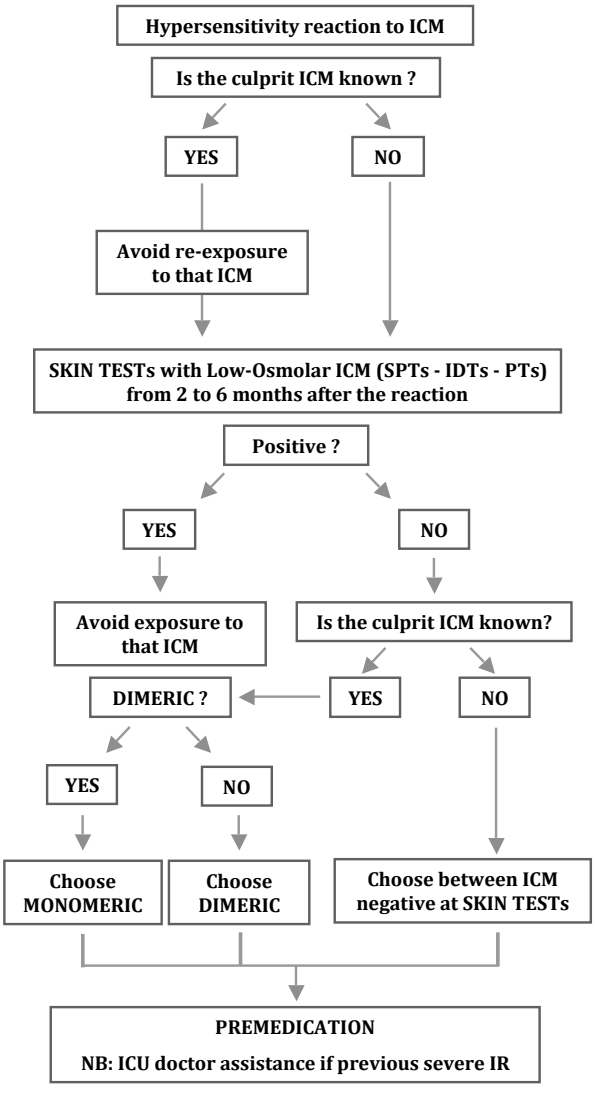
Algorithm for the selection of alternative iodinated contrast media. Alternative ICM for subsequent radiological procedures were chosen according to an algorithm based on skin tests results and patient history, proposed in **figure 1**: (i) avoidance of the previous culprit ICM, when known, was mandatory, even in presence of negative skin tests for that compound; (ii) contrast media with positive results on skin tests were avoided as well; (iii) in the absence of positive skin tests and/or known culprit ICM, non-ionic dimeric ICM were preferred over monomeric ICM, because the former are typically less implicated in immediate life threatening reactions (6,7); (iv) in the presence of positive skin tests for all the tested ICM, especially when prior hypersensitivity reaction was severe, iodinated contrast enhanced exam was discouraged, and a possible alternative procedure was suggested.

Premedication. Premedication was adopted in all patients with a previous history of hypersensitivity to ICM undergoing a new radiological examination, regardless the entity of the initial reaction. Premedication was performed according to a protocol approved and adopted by the American College of Radiology for the last 5 years: Methylprednisolone (Medrol®) 32 mg by mouth 12 hours and 2 hours before ICM injection, and Hydroxyzine Hydrochloride (Atarax®) 25 mg by mouth 1 hour before ICM injection (22). Intensive care unit doctor assistance was requested when the first hypersensitivity reaction was immediate and life threatening.

Follow-up. After ICM re-exposure, patients were monitored for one hour and discharged if no IRs occurred. Patients were then instructed to report any type of NIR occurring in the following 7 days, and to take pictures of eventual non-immediate skin eruptions; moreover, in case of adverse events occurring in this time frame, patients were immediately evaluated at our Institutes. Finally, patients were called a week after ICM injection and asked for possible hypersensitivity reactions, clinical conditions and drug assumption (mainly corticosteroids and/or antihistamines) in the previous 7 days.

Statistics. Statistical analysis was performed using GraphPad Prism software 6.0. Continuous variables are expressed as mean (range minimum-maximum value), unless otherwise specified.

Figure 1 *- Algorithm for the selection of an alternative ICM.*



Iodinated Contrast Media: ICM, Skin Prick tests: SPTs, Intradermal tests: IDTs, Patch tests: PTs, Immediate reaction: IR.

Results

Clinical characteristics of the patients' cohort

Thirty-six patients (mean age 58 years; range 22-75) (9 males and 27 females) were included in this study. Their clinical characteristics are summarized in **table 1** and **2**. Nineteen subjects (mean age 58 years; range 22-75) experienced an IR; seventeen patients (mean age 57 years; range 35-75) had a NIR. The overall male:female ratio was 1:3, with an increased incidence of both IRs and NIRs among females. Adverse reactions of both immediate and non-immediate type were related to computed tomography (CT) scan in the majority of cases. The remaining cases of adverse reactions occurred during angiography (two cases of Irs and one case of NIR) and urography (one case of IR). The culprit ICM was known in 27/36 cases (75%) (14 cases of Irs and 13 cases of NIRs) and unknown in 9/36 cases (25%) (5 cases of Irs and 4 cases of NIRs): Iopromide was the most frequently involved ICM both in Irs and NIRs.

An allergic background was present in 6/19 patients (32%) who experienced an IR and 7/17 patients (41%) with a previous NIR. Drug hypersensitivity represented the most frequently reported past allergic manifestation in both groups, followed by rhino-conjunctivitis and allergic contact dermatitis. Sixty-eight percent and 59% of subjects with immediate and non-immediate reactions, respectively, were not allergic. Seventy-five percent of the patients who experienced an adverse reaction to ICM in our cohort (14/19 patients with previous Irs and 13/17 with NIRs) had an underlying oncological disease and required periodical follow-up radiological exams.

Table 2 summarizes clinical manifestations of Irs and NIRs to ICM in the patients' cohort. Immediate reactions consisted of 12 grade 1, 3 grade 2 and 4 grade 3 reactions; NIRs were mild and moderate in 16 and 1 cases, respectively. Mucocutaneous involvement was the presenting feature in 95% of Irs and 100% of NIRs. Respiratory symptoms manifested only as part of an IR in 37% of patients. Gastrointestinal and neurological involvement accounted for a minor proportion of allergic manifestations. Four subjects (11%) experienced anaphylactic shock requiring epinephrine injection.

Skin testing

Skin tests were performed on average 16 weeks (range 8-25) after the reaction, and results are reported in **table 3** and **4**. SPTs were negative in all patients. IDTs were positive in 7/19 patients with a previous IR and 4/17 patients with a previous NIR. In

Table 1 *- Clinical characteristics of the patients' cohort.*

	Immediate Reactions	**Non-immediate Reactions**	**Total**
Number of Patients	19	17	36
Female, n (%)	14 (74%)	13 (76%)	27 (75%)
Age, mean (range)	58 (22-75)	57 (35-75)	58 (22-75)
Allergic history, n (%)	6 (32%)	7 (41%)	13 (36%)
Rhinoconjunctivitis	3	2	5
Drug allergies	4	2	6
Allergic contact dermatitis	2	1	3
Food allergy		1	1
Hymenoptera venom allergy		1	1
Not allergic, n (%)	13 (68%)	10 (59%)	23 (64%)
Ongoing disease requiring ICM exam, n (%)			
Oncological disease	14	13	27 (75%)
Chronic pulmonary disease	2		2 (5%)
Cardiovascular disease	1	4	5 (14%)
Autoimmune disease	1		1 (3%)
Other	1		1 (3%)
Implicated contrast medium, n			
Iomeprol (non-ionic monomer)	2	2	4 (11%)
Iopamidol (non-ionic monomer)	1		1 (3%)
Iopromide (non-ionic monomer)	8	6	14 (39%)
Iodixanol (non-ionic dimer)	3	5	8 (22%)
Unknown	5	4	9 (25%)

the group of patients who experienced an IR, IDTs were positive in 2 cases with ICM diluted 1:100, 4 cases with ICM diluted 1:10, and 2 cases with ICM diluted 1:1; 6 subjects developed a skin reaction at 20 minutes (immediate reading) and 2 at 48 hours (delayed reading). In the group of patients who experienced an NIR, IDTs were positive in 4 cases with ICM diluted 1:10; two subjects developed a skin reaction at 20 minutes and 2 at 48 hours. PTs were positive at 48 hours in one patient with a previous NIR. The rate of positive skin tests in our cohort was 7/19 (37%) in the group of patients with a previous IR and 5/17 (30%) in the group of patients with a previous NIR. The overall rate of positive skin tests in our cohort was 12/36 (33%). When known, the culprit ICM elicited a positive skin test in 5/14 cases of IRs, and 2/13 cases of NIRs.

Re-exposure to iodinated contrast media

All patients underwent a new contrast enhanced radiological procedure. Patients with previous IRs and NIRs were re-exposed to the alternative ICM on average 8 months (range 1-12 months) and 5 months (range 1 week-21 months), respectively, after the index adverse event (**table 3** and **4**). All patient were premedicated, and ICM dosage was not adapted because of the past clinical history of adverse reaction. 18/19 patients with previous IRs (95%) and 17/17 patients with previous NIRs (100%) tolerated

Table 2 *- Adverse reactions to ICM: severity of the reaction and clinical manifestations.*

	Immediate Reactions n = 19	**Non-immediate Reactions n = 17**	**Total n = 36**
Severity of the reaction			
Grade I	12		
Grade II	3		
Grade III	4		
Grade IV			
Mild		16	
Moderate		1	
Severe			
Mucocutaneous involvement, n (%)	18 (95%)	17 (100%)	35 (97%)
Urticaria	6	6	
Mucocutaneous Angioedema	6		
Exanthema	1	11	
Erythema	4		
Conjunctivitis	1		
Respiratory involvement, n (%)	7 (37%)		7 (19%)
Rhinitis	2		
Dispnea	1		
Bronchospasm	1		
Laryngeal edema	3		
Gastrointestinal involvement, n (%)		2 (12%)	2 (5%)
Nausea/vomiting		2	
Neurological involvement, n (%)	1 (5%)		1 (3%)
Paresthesia	1		
Anaphylactic Shock*, n (%)	4 (21%)		4 (11%)

*Anaphylactic Shock was defined according to international Consensus statements[23].

the alternative ICM selected according to the previously cited criteria, reported in the algorithm in **figure 1**. A single patient, who had a previous grade 1 IR to an unknown ICM, developed a self-limited localized slightly itchy erythema 48 hours after exposure to iobitridol. Notably, according to our algorithm, a non-ionic dimeric ICM would have been the alternative of choice in this case; however, non-ionic dimers were not available when skin tests were performed and were, therefore, not tested.

Discussion

The incidence of hypersensitivity reactions to ICM has increased along with the large use of these compounds for both diagnostic and interventional procedures (2). However, our knowledge about the implicated allergic and non-allergic mechanisms has not grown in parallel and clinicians actually lack accurate techniques for the diagnosis of hypersensitivity to ICM. Moreover, the introduction of nonionic low-osmolar products drastically reduced life-threatening reactions but did not prevent them, and anaphylaxis still remains a major concern both for patients and radiologists. This is particularly true for clinical conditions where repeated ICM injections are required for evaluation or follow-up, as in case of neoplastic, cardiovascular or chronic inflammatory disorders. Indeed, in these common clinical settings, allergists are oftentimes asked to readily provide an

Table 3 *- Outcomes of patients with previous IRs re-exposed to alternative ICM.*

Pt.	Radiological procedure	Type of IR	Culprit ICM	Skin test results1	Alternative ICM	Months after reaction	Outcome
1	CT scan	Grade 1	Iodixanol	IDT 1:10 Iodixanol at 48 hrs	Iopromide	3	No reactions
2	CT scan	Grade 1	Unknown	Negative	Iodixanol	4	No reactions
3	CT scan	Grade 1	Unknown	Negative	Iodixanol	7	No reactions
4	Urography	Grade 1	Unknown	Negative	Iodixanol	6	No reactions
5	CT scan	Grade 1	Unknown	Negative	Iobitridol	11	NIR (Mild)
6	CT scan	Grade 1	Iomeprol	Negative	Iodixanol	5	No reactions
7	CT scan	Grade 1	Iopromide	Negative	Iodixanol	9	No reactions
8	CT scan	Grade 1	Iopromide	IDT 1:100 Iopromide	Iodixanol	2	No reactions
9	CT scan	Grade 1	Iopromide	IDT 1:10 Iopromide and 1:1 Iomeprol. IDT 1:1 iomeprol at 48hrs	Iodixanol	6	No reactions
10	CT scan	Grade 1	Iopromide	Negative	Iodixanol	3	No reactions
11	CT scan	Grade 1	Iodixanol	IDT 1:1 Iopromide	Iomeprol	1	No reactions
12	CT scan	Grade 2	Iopromide	Negative	Iodixanol	7	No reactions
13	CT scan	Grade 3	Iopromide	Negative	Iodixanol[2]	9	No reactions
14	CT scan	Grade 3	Iopamidol	Negative	Iodixanol[2]	7	No reactions
15	CT scan	Grade 3	Iodixanol	Negative	Iopromide[2]	6	No reactions
16	CT scan	Grade 3	Iomeprol	IDT 1:10 Iomeprol and Iopromide	Iodixanol[2]	2	No reactions
17	Angiography	Grade 3	Unknown	IDT 1:10 Iopromide	Iodixanol[2]	12	No reactions
18	Angiography	Grade 3	Iopromide	Negative	Iodixanol[2]	9	No reactions
19	CT scan	Grade 3	Iopromide	IDT 1:100 Iopromide	Iodixanol[2]	10	No reactions

[1]Skin tests included IDTs and PTs: only positive results are reported.
[2]ICU doctor assistance was requested in presence of a history of severe IR to a previous ICM. All patients underwent premedication before re-exposure to the alternative ICM.
Computed tomography: CT; Iodinated contrast medium: ICM; Immediate reaction: IR; Intradermal test: IDT.

alternative ICM, because radiological exams need to be repeated every 6 to 12 months.

For these reasons, premedication actually represents the most widely adopted measure for preventing recurrent hypersensitivity reactions to ICM. However, several works demonstrated that current premedication procedures appear to reduce symptoms, but may not prevent repeated reactions (16-19). Moreover, studies performed by Greenberger and colleagues found that repeated reactions to ICM decreased from 17-30% to 11% by using a corticosteroid and antihistamine preparation, but were not abolished (18). In other words, premedication alone has been proven to be insufficient for a complete prevention of recurrent reactions to ICM, and we are actually unable to predict those patients that will react despite pretreatment.

International guidelines also suggest avoidance of the culprit ICM as an additional preventive measure (4), but the causative

Table 4 *- Outcomes of patients with previous NIRs re-exposed to alternative ICM.*

Pt.	Radiological procedure	Type of NIR	Culprit ICM	Skin test results[1]	Alternative ICM	Months after reaction	Outcome
1	CT scan	Mild	Iodixanol	Negative	Iopromide	2	No reactions
2	CT scan	Mild	Iomeprol	Negative	Iodixanol	7	No reactions
3	CT scan	Mild	Unknown	Negative	Iodixanol	9	No reactions
4	CT scan	Mild	Iopromide	Negative	Iodixanol	6	No reactions
5	CT scan	Mild	Iopromide	Negative	Iodixanol	3	No reactions
6	CT scan	Mild	Iopromide	IDT Iomeprol, Iodixanol, Iopromide 1:10 at 48 hrs	Iohexol	21	No reactions
7	CT scan	Mild	Iodixanol	IDT 1:10 Iodixanol at 48 hrs	Iopromide	8	No reactions
8	CT scan	Mild	Unknown	PT Iohexol at 48 hrs	Iodixanol	5	No reactions
9	Angiography	Mild	Iodixanol	Negative		6	No reactions
10	CT scan	Mild	Iodixanol	Negative	Iopromide	3 weeks	No reactions
11	CT scan	Mild	Iopromide	Negative	Iodixanol	4	No reactions
12	CT scan	Mild	Iopromide	Negative	Iodixanol	1 week	No reactions
13	CT scan	Mild	Iopromide	Negative	Iodixanol	2 weeks	No reactions
14	CT scan	Mild	Iodixanol	Negative	Iopromide	1	No reactions
15	CT scan	Moderate	Unknown	IDT 1:10 Iopromide	Iodixanol	7	No reactions
16	CT scan	Moderate	Unknown	IDT 1:10 Iopromide	Iodixanol	4	No reactions
17	CT scan	Moderate	Iomeprol	Negative	Iodixanol	8	No reactions

[1]Skin tests included IDTs and PTs: only positive results are reported. All patients underwent premedication before re-exposure to the alternative ICM. Computed tomography: CT; Iodinated contrast medium: ICM; Non-immediate reaction: NIR; Intradermal test: IDT; Patch test: PT.

iodinated compound is still rarely reported by radiologists and, thus, typically ignored in everyday clinical practice.

Thus, different approaches have been evaluated in order to identify safe alternative ICM. For instance, drug provocation test was reported to reliably diagnose NIRs to ICM, and was proposed as an additional tool for identifying alternative compounds, although reasonable safety concerns still remain to be completely addressed (24,25). Similarly, a large European multicentric study reported high specificity of skin tests in the diagnosis of immediate and non-immediate ICM reactions, but their usefulness in the selection of alternative and safe compounds was not evaluated (15). In trying to address this issue, a recent pivotal study reported a negative predictive value for ICM skin tests of 97%; of note, patients in this series were not premedicated before re-exposure to ICM (26). However, this promising result has to be interpreted in light of two main limits: (i) the absence of reports about the culprit ICM, and (ii) the long median time interval between the reaction and skin tests (11.5 years). In effect, since avoidance of the culprit ICM reduces the likelihood of a second hypersensitivity reaction, awareness of the implicated substance should integrate skin tests' results for deciding which alternative compounds to inject. On the other hand, skin tests with ICM should be performed from 2 to 6 months after the reaction in order to obtain the highest sensitivity (4,15). At later time points, in fact, loss of sensitization is known to significantly decrease the frequency of positive responses (15,27). Moreover, the negative predictive value of skin tests with ICM was calculated by analyzing immediate and non-immediate reactions together, although differences in terms of pathogenic mechanisms between the two types of reactions are well known in the literature (9-14).

All in all, standardized guidelines for preventing recurrent adverse reactions to ICM are lacking, adequate tools for the selection of safe alternative iodinated compounds need to be refined,

and premedication alone does not offer complete protection to patients with hypersensitivity to these substances.

In the present work, we validated an algorithm that efficiently protected 95% of patients with a previous IR and 100% of patients with a previous NIR from recurrent reactions to ICM. The algorithm was based on (i) the avoidance of the culprit ICM (when known in the majority of cases), (ii) the selection of an alternative ICM by integrating clinical history and skin tests results, and (iii) the premedication of all patients. Of note, all patients underwent skin tests within 2 to 6 months after the index adverse reaction, in accordance with the international guidelines (4,15).

Thanks to this approach, we were able to safely re-expose all patients to ICM early after the index adverse event, because the vast majority of subjects in our cohort had clinical priorities related to follow-up of oncological or chronic inflammatory diseases. The algorithm was equally efficacious both in case of a previous immediate and non-immediate adverse reaction to ICM. In particular, the combined use of SPTs, immediate and delayed reading IDTs and PTs, guided the selection of an alternative compound, both in case of positive and negative result. In fact, positive skin tests identified ICM that were avoided in subsequent radiological exams, while negative skin tests identified potentially safe ICM, unless involved in the previous adverse reaction. The knowledge of the culprit ICM was, therefore, crucial in the algorithm, since that substance was avoided in the following radiological procedures. Hence, radiologists are strongly encouraged to record the name of the injected iodinated compound, especially if patients need to undergo repeated exams. In light of these considerations, it is reasonable to think that the false negative patient had, indeed, a second adverse reaction to the same ICM, because the initial culprit ICM was unknown.

Moreover, it is noteworthy to observe that the proportion of patients who tolerated the alternative ICM (95% of patients with a previous IR and 100% of those with a previous NIR) was very similar to what reported by Caimmi without premedication. Of course, it is possible that premedication might have suppressed part of minor reactions in our cohort of patients. Alternatively, since both immediate and non-immediate reactions are known to occur despite pretreatment with corticosteroids and antihistamines, we might speculate that the selection of the alternative ICM, rather than premedication, played a major role in preventing recurrent hypersensitivity reactions. A third additional and comprehensive hypothesis is that both the selection of an alternative ICM and premedication concurred in efficaciously protecting patients from a subsequent adverse reaction.

Finally, the presence of positive skin tests results for ICM other than the culprit ones, might indicate either a real multiple sensitization, or cross-reactivity between different iodinated compounds. Indeed, cross-reactivity between different ICM is a clinically important problem and a well-defined phenomenon that primarily resides in the presence of contrast media-specific T cells (14). In this sense, skin tests might have been of further help in identifying potentially additional harmful ICM for individual patients.

Dissecting the protective contribution of premedication as well as the negative predictive value of skin tests is far beyond the purposes of the present study, but the reported results are in accordance with those of the literature and support the notion that premedication alone is not sufficient to control further reactions to ICM. Rather, the identification of a safe alternative ICM appears to be equally important for reducing the likelihood of a new hypersensitivity reaction.

Conclusions

In conclusion, we herein provide clinicians with a practical algorithm for approaching patients who need to be re-exposed to ICM despite a history of immediate or non-immediate hypersensitivity reactions to iodinated compounds. In particular, given the lack of established guidelines for the management of these subjects, the proposed algorithm represents a reliable and easily replicable tool for safely re-exposing patients to ICM in clinical settings where repeated radiological examinations are required.

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Anaphylaxis: a one-year survey on Medical Emergency Service in Liguria (Italy)

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Summary
Anaphylaxis is a severe, life-threatening, generalized or systemic hypersensitivity reaction. The diagnosis is mainly based on clinical ground. This study aimed at evaluating the records of phone calls and medical visits for anaphylaxis occurred in Region Liguria during 2013. The phone call is managed in each headquarter, and classified according to a level of care intensity and a presumed level of criticality, according to established criteria. Criticality is then re-evaluated (detected criticality) at the end of medical visit, following the same score adding the black code defining died patients. Most of the phone calls (553) to the MES were recorded in summer (37.4%). Anaphylaxis was confirmed in about half of patients. There was a fair agreement between presumed and detected criticality ($k = 0.322$, $p < 0.001$). In addition, 530 patients (95.8%) were transported to Emergency Room. In conclusion, the present study shows that anaphylaxis represents a serious and relevant medical problem in the general population at any age, and should always be carefully managed.

Introduction

Anaphylaxis is a severe, life-threatening, generalized or systemic hypersensitivity reaction (1). However, there is no definitive consensus about definition and diagnostic criteria. The most quoted work definition was proposed by Sampson and colleagues: anaphylaxis is likely when any of 3 criteria are fulfilled: i) acute onset of an illness with involvement of skin/mucosal tissue and airway compromise or reduced blood pressure or associated symptoms; ii) 2 or more of the following after exposure to known allergen for the patient: history of severe allergic reaction, skin/mucosal tissue, airway compromise, reduced blood pressure, gastrointestinal symptoms (for food allergy); iii) hypotension after exposure to known allergen for the patient (2). In Europe, the anaphylaxis incidence ranges from 1.5 to 7.9 per 100.000 person-years, so approximately 0.3% of the population experience anaphylaxis in their lives (1). Foods, drugs, stinging insects, and latex are the most common triggers. The updated World Allergy Organization Guidelines focuses on anaphylaxis diagnosis and management (3). Infants and teenagers have increased vulnerability to anaphylaxis. Comorbidity with severe or uncontrolled asthma, mastocytosis, and concurrent use of some medications increase the risk of severe or fatal anaphylaxis. Food is the most important trigger in childhood (4). Food anaphylaxis typically occurs after ingestion, more rarely after skin contact or inhalation. Drug anaphylaxis is most frequent in adults, whereas insect stings anaphylaxis may affect all ages.

The diagnosis of anaphylaxis is mainly based on a clinical ground. The clinical approach considers the presenting signs and symptoms and should exclude other sudden-onset multi-systemic diseases. Fortunately, only few food kinds, mainly including egg, milk, peanut, fish, soybean, wheat, are usually cause of anaphylaxis in children and adolescents, whereas shellfishes, crustaceans, and fresh fruits are relevant in adults. Their

relevance also depends on dietetic habits, different within each country. In this regard, there are some studies that addressed this topic, also considering the presenting clinical feature (4-7). About medications, b-lactam antibiotics and non-steroidal anti-inflammatory drugs (NSAID) are the most relevant cause of drug anaphylaxis. Finally, the timing, the clinical features, and the presence of co-morbidities (mainly asthma) and co-factors (e.g., NSAID, ACE-inhibitors, alcoholic drinks, and exercise) should be carefully evaluated.

In Italy, a medical emergency service (MES) exists to manage territorial emergency. MES is widespread distributed and is active h24. Recently, a study has been carried out to evaluate the medical emergency calls requiring attention for asthma and COPD exacerbations among the population of the territory of Genoa (Italy) in an 8-year period (8). Therefore, this study aimed at evaluating the records of phone calls and medical visits for anaphylaxis occurred in the Region Liguria during 2013.

Materials and Methods

Liguria is a North-Western Italian Region with about 1.6 million inhabitants. Medical Emergency Service (MES) is widespread in the territory, with 5 centrals and 18 medical stations. The service is available everyday h24.

The calls for suspected anaphylaxis occurred during the whole 2013 were evaluated.

The phone call is managed in each headquarter, and classified according to a level of care intensity and a presumed level of criticality, according to established criteria (http://www.emergencydispatch.org/it). Care intensity is scored according to a level ranging from Omega (the less relevant) to progressively more severe (Alpha, Bravo, Charlie, and Delta), up to the most critical Echo. The Academy indications are: Alpha level considers not-urgent dispatch of a basic BLS unit; Bravo level urgent dispatch of a BLS unit; Charlie level not-urgent dispatch of an ALS unit; Delta level urgent dispatch of an ALS unit. The care intensity definition is based on specific issues, including: vital parameters assessment, airways basic evaluation, presence of thoracic external compression, bleeding control, etc for BLS; advanced airways assessment (endotracheal intubation), medication use, manual defibrillators use, etc for ALS (http://www.mattoni.salute.gov.it/mattoni/documenti/MDS_MATTONI_SSN_milestone_1.4.1_Classificazione_attivit_118_v1.0.pdf).

Presumed criticality is initially defined at the headquarters on the basis of a score based on colours: white (mild), green (moderate), yellow (severe), and red (life-threatening). Criticality is then re-evaluated (detected criticality) at the end of the medical visit following the same score, adding the black code defining died patients.

The supposed diagnosis of anaphylaxis was based on clinical criteria (1,2,3), such as: suggestive clinical history consistent with presenting symptoms, i.e. the demonstration of a cause/effect dependence between exposure to potential causal trigger and occurrence of anaphylaxis clinical features (*post hoc ergo propter hoc*). Cardiovascular features were: hypotension, impairment of conscious state, pale and floppy presentation; respiratory features were: breathlessness, tongue or throat swelling, throat tightness, stridor, talking difficulty, wheezing, cough, and tachypnea; gastrointestinal features were: vomiting, colic, and diarrhea; skin features were: angioedema, urticaria, itching, and erythema.

Statistical analysis

Epidemiological, demographic and clinical profiles of patients are expressed as count and percentage or mean and standard deviation. Any relationship between detected criticality or season during which the event occurred, was evaluated by a chi-square test for goodness of fit. A non-parametric Kruskal-Wallis test was performed to check for significant differences in age distributions of detected pathologies. Cohen's Kappa coefficient was used for assessing the degree of agreement between alleged and detected criticality and between alleged and detected pathology. A $p \leq 0.05$ was considered statistically significant. SPSS (IBM Corp.) v.20 was used for computation.

Results

Table 1 shows the demographic and clinical characteristics of patients reporting anaphylaxis. Globally, 553 calls occurred during 2013.

Most of the phone calls to the MES were recorded in summer (37.4%), followed by autumn (23.7%), spring (20.6%), and winter (18.3%). Two hundred and fifty-two patients (45.6%) were males, and the mean age was 43.09 ± 23.32 years. **Figure 1** shows the distribution of ages per number of cases.

The most frequently registered levels of care intensity were Alpha (36.7%) and Charlie (28.2%). Breathing and/or swallowing difficulty were reported in 120 (21.7%) patients. As for the distribution of presumed criticality, yellow score was the most frequent (46.8%) followed by green (26.6%) and red (26.0%). On the other hand, about the detected criticality yellow score was the most frequent (49.9%), followed by green (44.8%).

Cohen Kappa value indicated a fair agreement between presumed and detected criticality ($k = 0.322$, $p < 0.001$). On 546 criticalities, 311 (56.96%) showed an exact correspondence between presumed and detected, 218 (39.93%) were presumed more serious than the real criticality verified, and 17 (3.11%) were presumed less serious than the real criticality detected (**table 2** and **figure 2**). The sub-analysis in children and adolescents showed super-imposable results ($k = 0.35$, $p < 0.001$).

In particular, 15 patients on 17 with presumed red code were confirmed as red level (88.2%), whereas only 2 were assessed as yellow code (11.8%).

There was no death for anaphylaxis during 2013 in Liguria. However, we cannot exclude that some case of death for anaphylaxis occurred in the Region during the period of observation as not registered through MES.
Finally, 530 patients (95.8%) were transported to Emergency Room.

Figure 1 *- Distribution of ages per number of cases.*

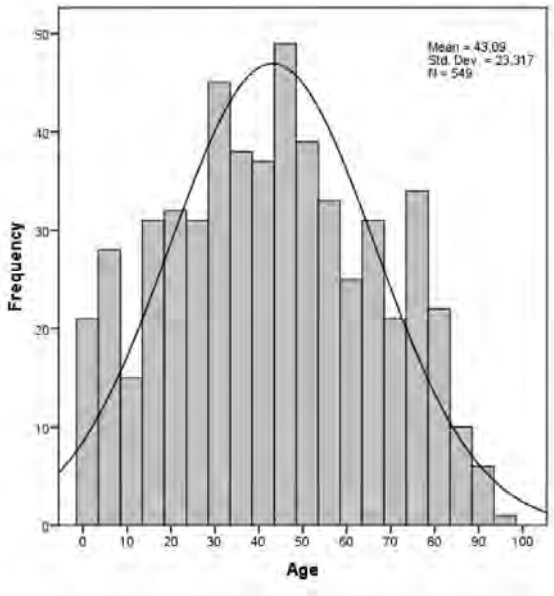


Table 1 *- (N = 553) - Descriptive analysis of the sample. Data are expressed as mean ± standard deviation and count (frequency).*

Season	*Spring*	114 (20.6)
	Summer	207 (37.4)
	Autumn	131 (23.7)
	Winter	101 (18.3)
Males, n (%)		252 (45.6)
Age (yrs)		43.09 ± 23.32
Care intensity level	*Alpha*	203 (36.7)
	Bravo	101 (18.3)
	Charlie	156 (28.2)
	Delta	91 (16.5)
	Echo	2 (0.4)
Presumed criticality	*White*	1 (0.2)
	Green	147 (26.6)
	Yellow	259 (46.8)
	Red	144 (26.0)
Detected criticality	*White*	7 (1.3)
	Green	248 (44.8)
	Yellow	276 (49.9)
	Red	17 (3.1)

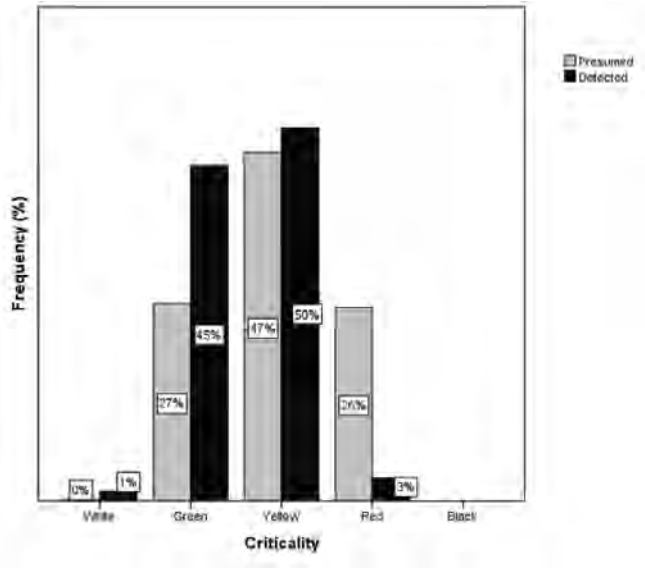
Table 2 *- Agreement between presumed and detected criticality.*

		Detected criticality				**Total**
		White	**Green**	**Yellow**	**Red**	
Presumed criticality	White	*1 (100.0)*	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
	Green	1 (0.7)	*130 (89.0)*	15 (10.3)	0 (0.0)	146 (100.0)
	Yellow	1 (0.4)	88 (34.4)	*165 (64.5)*	2 (0.8)	256 (100.0)
	Red	4 (2.8)	29 (20.3)	95 (66.4)	*15 (10.5)*	143 (100.0)
Total		7 (1.3)	247 (45.2)	275 (50.4)	17 (3.1)	546 (100.0)
Measure of agreement		k = 0.322; p < 0.001				

Table 3 *- Agreement between presumed and detected criticality in 86 minors.*

	Presumed criticality				
	White	Green	Yellow	Red	Total
age		7.97	7.84	8.84	
	0	29	38	19	86
	Detected criticality				
	White	Green	Yellow	Red	Total
age		8.95	6.98	11.0	
	1	41	42	2	86
Measure of agreement		k = 0.35; p < 0.001			

Figure 2 *- Presumed and detected criticality for anaphylaxis episodes.*



Discussion

The present survey demonstrates some interesting findings. Firstly, the highest frequency of phone calls for anaphylaxis occurred during summer (37.4%) and autumn (23.7%), such as about 2/3 of the global sample. This fact might be dependent on the prevalence of outdoor living in these seasons and the abundance of triggers, such as fruits and insects.

Secondly, mean age is nearly corresponding to half the survival rate. Indeed, anaphylaxis may occur at any age. This outcome is particularly relevant from a clinical point of view. Anaphylaxis should be always considered at any age.

Thirdly, anaphylaxis was confirmed in about half of cases, with a fair concordance between presumed and confirmed diagnosis, corresponding to red and yellow scores. Particularly, it is to note that there is a trend to overestimate the clinical severity by patients or observers. In fact, the severity of the red code was confirmed only in about 1/10 of cases. On the other hand, about half of calls corresponded to less severe allergic or non-allergic reactions. However, almost all subjects (95.8%) referred to the Emergency Room. This aspect underlines the relevance that this issue deserves.

The limitations of this study are the lack of details concerning the clinical presentation and the lack of triggers definition, in other words a definitive and correct diagnosis of anaphylaxis. These shortcomings depend on the particularity of medical records used by MES and, of course, on the peculiarity of MES deputed to emergency care. In fact, it has to be considered that there is a relevant diagnostic difficulty of this clinical picture during a MES intervention. In addition, several disorders should be considered in differential diagnosis, e.g. syncope, hearth infarction, stroke, vagal hypertonia, etc. Moreover, considering these limitations, mainly lack of details concerning clinical presentation and triggers definition, anaphylaxis can be only suspected and not confirmed, because diagnostic methods are very limited in the context of the emergency interventions.

On the other hand, the studies conducted in Italy about anaphylaxis were addressed to specific causes of anaphylaxis, such as food or hymenoptera allergic reactions, or concerned the experience of single Emergency Department. Therefore, the present study represents a further demonstration of the MES utility in

epidemiologic studies about several acute clinical pictures.
Obviously, further studies should be conducted addressing the limitations of the present survey.
In conclusion, the present study shows that anaphylaxis represents a serious and relevant medical problem in the general population at any age and should always be carefully managed.

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Overcoming recurrent spontaneous abortions in women suffering from IgG subclass deficiency: high efficiency of low dose intravenous immunoglobulins treatment

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Key Words

Idiotype-antiidiotype network restoration in pregnancy; IgG subclasses unbalance in habitual abortions; restoring maternal-foetus immunologic tolerance

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Summary

Problem. *It's well known that iv. immunoglobulins may be useful to overcome habitual abortions, but the mechanisms at the base of a successful outcome and the likelihoods are still unknown.* ***Method of study****. In one hundred and sixty women with habitual abortions and one hundred and sixty healthy mothers, we evaluated blood IgG subclasses; among the patients, sixteen merely showed IgG subclass deficiency, after leaving out any autoimmunity and/or coagulation disorders. All the patients (100%) showed IgG3, twelve (75%) IgG1, eight (50%) IgG4 and six (37,5%) IgG2 deficiency; healthy control people's IgG subclasses fell in normal range in 156 women, but just four women showed IgG2 and IgG4 deficiency with neither immune deficiency's clinical marks nor increased vulnerability to infections. All the patients were treated with whole immunoglobulins iv. infusion (200 mg/kg/monthly) all over the pregnancy.* ***Results.*** *The successful pregnancy rate is very high (> 90%): 100% out of women showing IgG1 (12/12), 87,5% of IgG3 (14/16), 75% of IgG4 (6/8) and 66% of IgG2 deficiency (4/6) had successful pregnancies. The Odd's Ratio between IgG subclass deficiency and recurrent abortions is 4,33 with confidence interval of 95%; chi square value is 7.68 ($p < 0.025$).* ***Conclusions.*** *Low dose immunoglobulin infusion is the only effective way to reach successful pregnancy, despite previous habitual abortions in patients suffering from IgG subclass deficiency without autoimmunity and/or coagulation disorders, likely restoring idiotype-antiidiotype network; showing evidence of IgG subclasses deficiency (mostly IgG1 and IgG3) may help identify patients who can benefit from iv. immunoglobulin treatment.*

Introduction

Habitual pregnancy loss may be determined both by foetus and/or mother related causes; as to foetus-related, the main causes are genetic (aneuploidy); as to mother-related, the main causes are genetic, abnormal uterine structure, hormones, toxic exposure, malnutrition, metabolic, coagulation and/or immunologic disorders (1,2). Regarding immunologic disorders, both organ and non-organ specific autoimmune diseases, as well as immunodeficiencies, may lead to a pregnancy loss (3); the mechanisms leading to habitual abortions in case of immunodeficiencies may be very different. An anti-idiotypic antibodies unbalance may lead to overthrow both the maternal tolerance against semiallogeneic trophoblast cells (4,5,6) and the right myometrium tone (7). In fact, it has been shown (4) that the sera of women undergoing habitual abortions hold antibodies playing anti-idiotypic activity (8,9); moreover, a whole idiotypic network is essential to attain a correct blastocysts implantation and to overcome the rejection against trophoblast cells (10). On the other hand, the whole idiotypic network is fundamental to ensure a correct uterine contractility (11,12); the ability to bind idiotypes is related to higher molecule's flexibility (13) and this is a IgG1 and IgG3 peculiar property (14). This serendipitous study started in 2003, when the case of a 34 year-old woman who underwent five abortions without any apparent clear cause (the search for hyperhomocysteine, co-

agulation abnormalities, autoimmunity and/or LAC activity was negative) occurred to our observation. The immunologic characterization didn't show any cellular abnormality but only IgG1 and IgG3 deficiency, as referred to healthy controls. We decided to treat her with a whole immunoglobulins low dose iv., and the woman happily carried on her sixth pregnancy (15).

Materials and methods

Blood IgG subclasses of one hundred and sixty women having clinical history of more than 2 spontaneous abortions and one hundred and sixty healthy mothers without any history of abortions as control people were evaluated by nephelometry (Beckman). In the patients suffering from IgG subclass deficiency (16/160), the abortions occurred both very early, starting from the fourth week, and later, till the twentieth week; the symptoms were bleeding and/or myometrium contraction resistent to all tocolytic drugs. The search for organ and non organ-specific autoimmune diseases, including Hughes Syndrome (antids-DNA, antiENA, P-and C-ANCA, antinuclear autoantibodies, IgG and IgM antibeta2glycoprotein 1, IgM and IgG antiphospholipid and anticardiolipin autoantibodies, Lupus anticoagulant), hyperhomocysteinemie and coagulation diseases (C and S Prot., P.T., aP.T.T., D-Dimer) was performed. Since the early b-HCG increase all the patients showing sole IgG subclass deficiency were treated with whole immunoglobulins iv. infusion (200 mg/kg/monthly) all over the pregnancy in order to restore humoral immunity, hoping to gain a successful pregnancy; this low therapeutic dose has been shown to be safe and effective in treating unexplained recurrent spontaneous abortions by increasing the blood s-HLAG and IL-10 tolerogenic cytokine in a prospective clinical trial (16), while higher doses (0,4-1 g/kg) are indicated in habitual autoimmune disease-driven abortions (17,18,19). The study was approved by local ethical committee and informed consent was asked and attained by the patients. No placebo control therapeutic intervention was planned because of ethical reasons; on the other hand, the patients hadn't had any benefit from previous treatments with conventional therapies (hormones, tocolytic drugs). Statistical analysis was performed using chi square test and Odd's ratio.

Results

Among one hundred and sixty patients just sixteen 24-42 year-old women (10%) showed IgG subclass deficiency: all of these (100%) showed IgG3, twelve (75%) IgG1, eight (50%) IgG4 and six (37.5%) IgG2 deficiency. As to one hundred and sixty healthy control people, just four (2.5%) showed IgG subclasses deficit, involving IgG2 and IgG4 only, without any clinical symptom, while one hundred and fifty-six women had IgG subclasses falling under normal range as referred to more than eight year-old people (**table 1**). The statistical association between IgG subclass deficiency and habitual abortions significantly reaches an Odd's ratio of 4.33 with confidence interval 95%, while chi square test is 7.68 ($p < 0.025$) (**table 2**). Among the patients' group, symptoms resistant to every other therapy, symptoms consisting both in early bleeding, and later incoercible uterus contractions, were fully bridled thanks to iv. immunoglobulins treatment. A successful pregnancy was reached by all (100%) patients showing IgG1 deficiency (12/12), 87.5% (14/16) out of those with IgG3 deficit, 75% (6/8) with IgG4 deficit, and 66% (4/6) out of those showing IgG2 deficiency. No detrimental effect was registered either on mothers or on children.

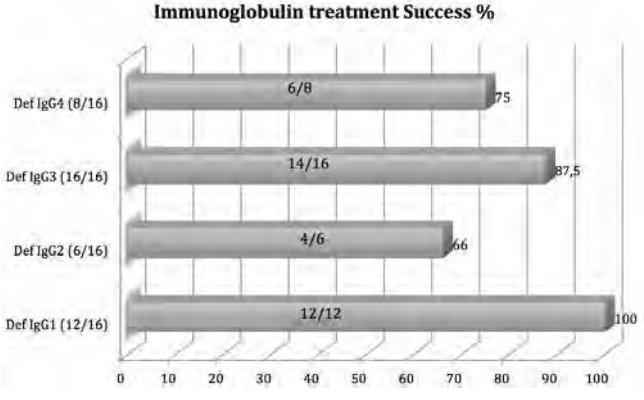
Table 1 *- IgG subclass normal values (women more than 8 years-old).*

	Mg/dl	%
IgG1	580-700	60
IgG2	290-350	30
IgG3	68-82	7
IgG4	29-35	3

Table 2 *- Contingency Table between IgG subclass deficiency and habitual abortions: chi square = 7.68 (p < 0.025); Odd's ratio = 4.33 (confidence interval 95%).*

		Habitual abortions		Total
		+	-	
IgG subclass deficiency	+	16	4	20
	-	144	156	300
	Total	160	160	

Figure 1 *- Relationship between immunoglobulin treatment and successful pregnancy % in sixteen women suffering from IgG subclass deficiency with habitual abortions.*



Discussion

The prevalence of recurrent abortions (R.A.) in our hospital is about 1.5% (20/1420 gestations/year). The prevalence of IgG subclass deficiency in people suffering from R.A. is on average 10% (16/160 in the last eight years). Unexplained recurrent abortions have already been recorded as IgG subclass deficiency's clinical expression, as well as tooth decay, relapsing pharyngotonsillitis, H.P. infestation, mucocutaneous herpes, urticaria, asthma, heart's valves diseases, urinary tract infections (20). Abortions may occur both very early during pregnancy, from second to eighth gestation week, and also later, till the twentieth; in the former case, the first more common symptom is bleeding, in the latter, uncontrolled myometrium contractions; both these symptoms may be related to idiotypic network's unbalance. In a normal immunoglobulin structure, the idiotypic specificity inhabits the Fab portion of the molecule's frame; the idiotype's function and target binding ability may be modulated by a second anti-idiotype antibody able to bind idiotype's Fab; but also the anti-idiotypic antibody's function may be under- or up-regulated by a third anti-antiidiotype antibody. This idiotypic network may regulate the function of virtually every biologic receptor, including the myometrium muscarinic acethylcholine receptors; so, an immunologic unbalance of the idiotypic network may lead to uncontrolled myometrium contraction (21,22). The idiotypic network's unbalance may also overthrow both the immunologic recognition of antigens (auto-, allo- and iso-antigens) and the tolerance induction mechanisms, including the mother's immunologic tolerance against the foetus semiallogeneic hystocompatibility antigens. Moreover, during pregnancy the sole mother's immunoglobulin subclass entering foetus circulation is IgG1, thanks to the molecule's active carriage from mother to foetus' blood (23), so ensuring the right immunologic defence against endouterine infections (viruses, bacteria, parasites). The iv. Immunoglobulin treatment is known for bringing benefits among the patients undergoing habitual abortions (16,17); the invoked mechanisms go from natural killer cells down-regulation (24,25,26) to idiotypic network's restoration (18,19). Some reviews failing to find a benefit from iv. Immunoglobulins for treating unexplained recurrent miscarriage (27), don't consider the etiology of R.A., so that the heterogeneity of inclusion criteria may endanger conclusion's congruity.

Conclusions

The present study shows for the first time that: 1) IgG subclass deficiency (mostly IgG1 and IgG3) may lead to habitual abortions in about 10% of women having R.A.; 2) habitual abortions are included among the clinical features of IgG subclass deficiency; 3) the slow iv. infusion of low dose of whole immunoglobulins (200 mg/kg/monthly) all over the pregnancy since the first increase of β-HCG is the only effective treatment allowing to happily carry on the pregnancy in women having a clinical history of habitual abortion and isolated IgG subclass deficiency; 4) the successful pregnancy rate after this therapy in selected patients is very high (on the average > 90%). The IgG1 and IgG3 subclasses likely include most idiotype and antiidiotype antibodies, so that their deficiency may lead to idiotypic network's unbalance, allowing to overthrow maternal immunologic tolerance mechanisms against foetus semiallogeneic antigens and the right myometrium tone regulation during the pregnancy; the low dose of whole immunoglobulin treatment is the only effective way to overcome habitual abortions in women suffering from IgG1 and IgG3 subclass deficiency without autoimmunity and/or coagulation disorders.

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Severe bronchiectasis in a patient with common variable immunodeficiency

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Summary
Background. *Bronchiectasis are common in Common Variable Immunodeficiency. These patients are prone to infection, leading to progressive lung destruction and accelerated FEV1 decline.* ***Clinical case.*** *40 year-old man, with recurrent respiratory infections, autoimmunity and diarrhea since age 7. At 17 CVID was diagnosed and IVIgG was started. During the following years, respiratory symptoms progressively worsened and bronchiectasis was found on thoracic computed tomography. Bronchoscopy revealed Pseudomonas aeruginosa in bronchoalveolar lavage and bronchial secretions cultures. Eradication therapy led to clinical improvement.* ***Discussion.*** *This case report stresses the importance of regular microbiological screening and appropriate antibiotherapy. Early/aggressive treatment may significantly impact on patients' evolution.*

Introduction

Common variable immunodeficiency (CVID) is the most common primary immunodeficiency in adults, with a prevalence of 1/50 000 in Western Countries (1-4). Patients are diverse in regard to clinical presentation, that includes increased susceptibility to infection, autoimmunity, granulomatous disease and unexplained polyclonal lymphoproliferation (2).
Bronchiectasis are a common finding in CVID, reported in up to 29% patients (1,5,6). In contrast, *Pseudomonas aeruginosa* infection is seldom reported (4,7,8). The authors report a case of CVID and *Pseudomonas aeruginosa* infected bronchiectasis, illustrating the severity of this combination in CVID.

Case report

A 40 year-old man was referred to Immunoallergology Department due to recurrent respiratory infections. Since 7 year-old until adolescence, he had several hospital admissions due to hemolytic anemia, thrombocytopenia and pneumonia, occasionally complicated with pleural effusions. By age 15, the patient developed intermittent diarrhea and was diagnosed with terminal ileitis, requiring systemic corticosteroids for a short period, although total duration and doses were not possible to determinate. One year later, abnormal chest X-ray findings led to pulmonary surgical biopsy, which revealed lymphocytic interstitial pneumonitis.
By age 17, he was admitted due to meningitis and sepsis. Blood analysis revealed hypogammaglobulinemia (IgG 250 mg/dl [RV: 751-1560 mg/dl], IgA 45 mg/dl [RV: 82- 453 mg/dl], and IgM 77 mg/dl [RV: 46-304 mg/dl]). Secondary causes for hypogammaglobulinemia were excluded, and CVID was diagnosed. He started replacement therapy with IVIgG (0.5 g/Kg/month).
From age 22, diarrhea became persistent, with a mean of 2-3 daily liquid stools. *Giardia lamblia* was identified in different occasions and treated with metronidazole.

By age 38, despite regular IVIgG treatment and maintaining pre-infusion IgG serum levels around 600 mg/dl, he reported persistent cough and bronchorrhea requiring frequent antibiotic courses (> 6/year) and hospital admissions.

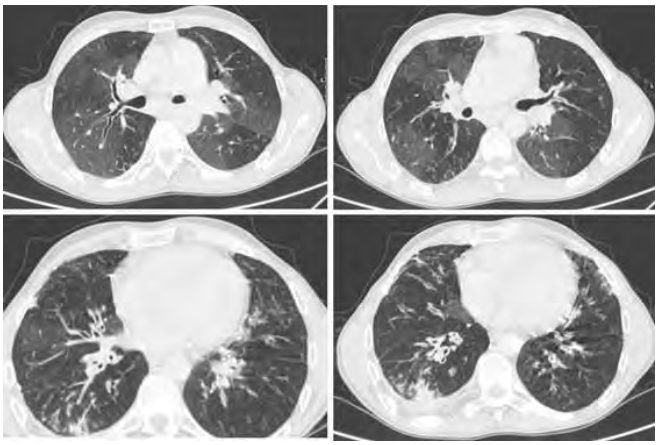
In his first physical examination in Primary Immunodeficiency clinic he presented low body mass index (BMI 17.9), tachypnea, diminished breath sounds, bronchospasm and rales in the inferior 2/3 of both hemithoraces. He also presented hepatomegaly and marked splenomegaly (palpable splenic notch by the medial line). Laboratory evaluation revealed serum IgG 1050 mg/dL (under IVIgG replacement), decreased IgA (4 mg/dl) and IgM (9 mg/dl); and zinc, iron and B12 vitamin deficits, as well as increased alkaline phosphatase, β2-microglobulin and angiotensin conversing enzyme serum levels. Fecal fat test and serum albumin were normal, and sweat test was negative. Bacterial and parasitological exams of stools were negative, and HIV1 and 2 antigens were not detected in serum. Lung function tests showed severe large and small airway obstruction and low carbon monoxide diffusion (**table I**). Thoracic CT-scan showed bronchiectasis and bronchiolectasis, more evident in the medium and lower lobes, bilateral mosaic pattern and multiple infra-centimetric ganglia in various chains (**figure 1**). Upper gastrointestinal tract endoscopy showed chronic pangastritis and atrophic duodenitis.

Immunophenotyping of peripheral blood lymphocytes revealed normal B cell counts with decreased frequency of switched-memory B cells and expansion of B cells expressing low levels of CD21 (CD21lo subpopulation), as well as CD4+ naive T-cells depletion and increased frequency of CD4+ and CD8+ T lymphocytes expressing memory and activation markers.

IVIgG replacement dose was increased (1.2 g/Kg/month) in order to achieve pre-infusion serum IgG levels around 1000 mg/dl. Respiratory care was optimized, based on inhaled therapy (salmeterol and fluticasone association 50/500 mcg *bid* and tiotropium 18 mcg *tid*), oral n-acetilcysteine 600 mg opd (once per day) and regular respiratory physiotherapy. He also started oral omeprazole and replacement therapy with zinc, iron, vitamins D and B12.

We observed noticeable improvement of gastro-intestinal complaints, although bacteriological and parasitological exams of stools were negative in different occasions. Due to progressive worsening of bronchial obstruction (**table 1**) and suppuration requiring frequent courses of antibiotics, bronchofibroscopy was performed and revealed abundant purulent secretions, and *Pseudomonas aeruginosa* was isolated in both bronchoalveolar lavage and bronchial secretions cultures. The patient was admitted for eradication therapy with ceftazidime 2 g *tid*, amikacin 1g opd and ciprofloxacin 750 mg *bid* for 2 weeks, in accordance with antibiogram, and discharged on inhaled tobramycin 300 mg bid in alternate 28-day courses during one year and daily respiratory physiotherapy, in addition to the previous therapy. During the first year on tobramycin he had 3 exacerbations of bronchorrhea requiring oral antibiotic. Bacteriological exam of bronchial secretions was negative in different occasions. One year later, prophylaxis with azithromycin 500 mg 3 days/week was started due to return of fatigue, cough and bronchorrhea. This regimen was held for a period of one year with global clinical improvement, increased exertion capacity, decreased cough and sputum and weight gain (BMI 20).

Figure 1 *- Toracic CT scan (38 years old): Bronchiectasis and bronchiolectasis (upper left), mosaic pattern (upper right), tree-in-bud (lower left).*



Discussion

According to the European Immunodeficiency Society (ESID), clinical diagnosis of CVID requires the presence of at least one of the following: increased susceptibility to infection, autoimmune manifestations, granulomatous disease, unexplained polyclonal lymphoproliferation or an affected family member with antibody deficiency (9). In addition, there must be a marked decrease in serum IgG (< 2SD of the normal levels for age) and IgA, with or without low IgM in at least 2 measurements, and at least one of the following criteria must be met: poor antibody response to vaccines, exclusion of other secondary causes of hypogammaglobulinemia, diagnosis after 4 years old, and no evidence of profound T cell deficiency (9). In a recent European multicentric CVID cohort including 2212 patients, the total frequency of clinical features was evaluated in 902. The most common complications reported were pneumonia (32%), autoimmunity (29%), splenomegaly (28%), bronchiectasis (23%), granuloma (9%) and enteropathy (9%) (1). Heterogenous immunological phenotypes may underlie the clinical variability in CVID. The EUROclass classification arised from a multicentric study which evaluated 303 CVID

Table 1 *- Summary of the patient's lung function tests.*

Date	FEV_1/FVC (%)	FEV_1 (%)	FVC (%)	FEF 50/75 (%)	RV/ TLC (%)	Raw (kPa*s/L)	DL_{CO}/ DL_{CO}/ VA (%)	pO_2/pCO_2
Before referral to PID clinic	69	34	40	22/27	177/75	0,44	54.7/118.4	69/38
PA bronchiectasis' infection	47	24	42	8/6	213/88	1,00	55.6/125.7	73.1/39.4
9 months after tobramycin	57	24	34	10/9	235/89	1.20	44.0/98.8	
Beguining of azythromycin	47	26	46	10/13	196/87	0.98	58.6/120.1	74.9/43.0
7 months after azitromycin	52	44	44	11/5	189/83	0.78	42.7/95.9	

Legend
DLco: carbon monoxide diffusion; FEF: forced expiratory flow; FEV1: forced expiratory volume in the 1st second; FVC: forced vital capacity; PA: *Pseudomonas aeruginosa*; Raw: airway resistance; RV: residual volume; TLC: total lung capacity.

patients in order to improve and unify previous smaller-based classification schemes (10). According to this classification, a significant decrease in switched memory B cells (smB-) was associated with splenomegaly and granulomatous disease (10). Also, lymphoproliferation was associated with transitional B cell (smB-Trhi) expansion (lymphadenopathy) and CD21low B cells expansion (splenomegaly) (10).

The patient we present was classified according to EUROclass (10) as B+; SmB-; Trnorm; 21lo. He presented normal B cell count (B+), as described for most CVID patients (6); low frequency of switched-memory B cells (SmB-), which has been associated with chronic pulmonary disease (11,12), granulomatous disease and lymphoid proliferation (splenomegaly) (13,14); and abnormal expansion of B cells with decreased expression of CD21 expression (21lo), that has been associated with splenomegaly (10), autoimmunity (14), higher number of respiratory tract infections (14) and chronic respiratory disease (14), all features that our patient displayed.

Bronchiectasis in CVID has been related to severe/recurrent respiratory tract infections, unregulated inflammation, low numbers of memory B cells and CD4+ T-cell count below 700/µl (15,16). Several reports have suggested that maintaining high serum IgG levels is associated with a cutback in the progression of lung deterioration and decrease in frequency of severe bacterial infections (1).

In a cohort of 89 adults with non-CF bronchiectasis and followed for 5.7 ± 3.6 years, *Pseudomonas aeruginosa* was found in 12% to 33% patients (17). In this same cohort, a significant number of idiopathic causes was reported (77%) and thus assigning a minimal percentage of bronchiectasis to hypogammaglobulinemia (1%) (17). *Pseudomonas aeruginosa* infection in patients with non-CF bronchiectasis has been associated with more severe and rapid radiologic and lung function decline, as well as with an increase in mortality (18).

The combination of antibiotics administered to our patient upon *Pseudomonas aeruginosa* isolation, has been recommended for treatment of severe exacerbations in patients with non-CF bronchiectasis (19). During the subsequent year, chronic suppression with tobramycin aimed to reduce the bacterial load and associated inflammation. Anti-inflammatory and immunomodulatory properties have been claimed to macrolides (19,20). In the case we present, treatment with azithromycin was associated with clinical improvement and important increase on FEV1 in sequential lung function tests (**table 1**). Both decrease in exacerbations, after *Pseudomonas*' eradication, and anti-inflammatory properties of azithromycin have possibly contributed to this favorable evolution. In a recent meta-analysis of randomized and controlled trials, Zhuo *et al*. analyzed the efficacy and safety of macrolide therapy in adults with non-CF bronchiectasis (21). The authors found that there was a significant reduction in pulmonary exacerbations in patients undergoing macrolide treatment in association with improvement in lung function (21) and quality of life as compared to placebo group (22).

Prophylaxis in non-CF-bronchiectasis is not consensual (2,19), since limited results have been reported on it, and even less on CVID. The potential development of resistance is another area of concern, particularly in patients with bronchiectasis who might be infected with *Mycobacterium* species. Therefore, when considering this therapy, careful exclusion of Mycobacteria infection should be undertaken (23).

In our patient, the therapeutic regime used led to eradication of *Pseudomonas*, since subsequent regular microbiological sputum analyses were consistently negative for *Pseudomonas* infection, two years after completing the antibiotic treatment.

Because of the severity of pulmonary structural changes and functional deterioration, we have considered the adequacy of pulmonary transplant in this case which is reported in very few cases in the literature, with an average survival of only 2 years (24,25-27). In conclusion, prompt diagnosis and IVIgG therapy might decrease the frequency of complications (6). Bronchiectasis is frequent in patients with CVID. Infection contributes to pulmonary destruction and accelerated lung function

decline. Timely and adequate treatment may prevent chronic colonization, as well as delay lung function deterioration.

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Immediate-type hypersensitivity reaction to Mannitol as drug excipient (E421): a case report

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Key words
Mannitol; Urticaria; excipient; Rhinitis; oral challenge test; Paracetamol; olive tree pollen

Summary
Allergic reactions to mannitol have been reported rarely, despite its widespread use as a drug and as a food excipient. This is the first case report in which oral mannitol induces an immediate type hypersensitivity as a drug excipient, in a 42 year old man affected by rhinitis to olive tree pollen. Unusual and undervalued risk factors for mannitol hypersensitivity are examined.

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to be the second most common cause of drug-induced hypersensitivity reactions with immunological and/or non immunological mechanisms. When clinical manifestations, particularly urticaria and angioedema, are induced by a single NSAID molecule, the reaction is supposed to be genuinely IgE-mediated, because of its high selectivity (1). However, sometimes hypersensitivity reactions may be induced by an excipient, a preservative or a dye contained in the pharmaceutical preparation. In this case, the diagnostic procedure is much more complex and elaborated. The presumptive diagnosis is fortuitously suspected because, for instance, patient realizes to tolerate the same drug packaged in a different formulation and assumed accidentally (2). We describe the case of a patient with an urticaria-angioedema syndrome after taking effervescent granular formulation of paracetamol. We demonstrated that the culprit of the adverse reaction was the mannitol added as a sweetener to paracetamol, not the drug itself.

Case report

A 42 year old atopic male patient came to our attention for a severe urticaria and angioedema at the Allergy and Clinical Immunology Service of Civil Hospital Vito Fazzi (Lecce, Italy). Symptoms appeared two hours after having taken 500 mg granular effervescent paracetamol packaged as sachets (Tachipirina® effervescent granules, Angelini Inc., Milan, Italy). Neither respiratory involvement nor hypotension were present, so the patient was promptly treated with corticosteroids and antihistamines orally, until complete remission of symptoms that occurred after three days. Maternal hypersensitivity to NSAIDs was reported in his clinical history, but he had not manifested any previous

adverse reactions to NSAIDs. He was also affected by a seasonal allergic rhinoconjunctivitis to Olive tree pollen (as confirmed by previous skin prick test already performed in another Allergic Service), treated with oral antihistamines during the May-June pollination period, classified as an intermittent severe rhinitis. Based on clinical manifestation and strict correlation between symptoms and drug intake, we considered paracetamol as the culprit agent for the adverse cutaneous reaction, and recommended patient to avoid its assumption. After one month, the patient underwent an oral incremental challenge test with alternative NSAIDs drugs (Nimesulide 100 mg and Etoricoxib 90 mg), which were well tolerated. Eighteen months later, the patient went back to our observation with a diffuse severe urticarial rash and facial angioedema associated to laryngeal stridor and shortness of breath. Symptoms appeared about 45 minutes after the ingestion of a cup of coffee sweetened with an industrial dietetic sugar-like product (Dietor®, Leaf Italia Inc., Bologna, Italy) that he had never taken before. We treated the patient administering systemic corticosteroids and antihistamines with prompt regression of symptoms. Patient denied assumption of any drug before the last episode. In the light of the new immediate-type hypersensitivity reaction, we decided to reconsider the previous diagnosis to carry out a more careful allergic investigation. Three weeks after the last reaction, skin prick test (SPT) with commercial inhalants extracts (Stallergenes Inc., Milan, Italy) for grass and tree pollens, animal danders, molds and house-dust mite were performed. The SPT confirmed the presence of monosensitization to the pollen of Olive tree previously reported by patient. Examination of Dietor® composition (a mix of sorbitol, mannitol and fructose) and Tachipirina® 500 mg formulation (Paracetamol 500 mg, NaH3CO, sodium carbonate 103.0 mg, citric acid 800 mg, mannitol 160.6 mg, sodium docusate 0,200 mg, maltilol 180.5 mg and aspartame 13 mg) evidenced the presence of mannitol in both compounds. Furthermore, patient had started again to drink coffee sweetened with normal sugar-cane, thus excluding the responsibility of coffee as an allergen. Then, a skin prick test with mannitol 20% (Isotol, Diaco Biofarmaceutici Industry, Trieste, Italy) was performed with negative result, while an intradermal test to mannitol diluted 1:10 with 0.9% sterile saline gave a positive response, resulting in a wheal with a diameter of 8 mm x 6 mm, while a SPT with histamine chloride 1%, as positive control, gave a wheal of 10 mm x 8 mm. Total serum IgE in addition to inhalants specific IgE were performed using the ImmunoCAP-System radioimmunoassay (Phadia Inc., Thermo Scientific, Uppsala, Sweden). Increased total IgE 154 IU/ml and Olive pollen specific IgE at 1.83 $kUAL^{-1}$ (cut-off value 0.10 $kUAL^{-1}$) were found. After having obtained the patient's written informed consent, an oral challenge test (OCT) was performed with Paracetamol 1000 mg tablets, which did not contain mannitol. Paracetamol was divided in 4 doses (250 mg) and administered orally with one hour intervals between each other .
During the OCT, arterial pressure, pulse-oxymetry and FEV1 were monitored (30 minutes and every hour after administering each dose or as soon as any symptoms arose). Patient remained in the hospital under medical supervision for at least two hours after the end of OCT, and then he was asked to contact doctors in the following 24 and 48 hours, in case any delayed reaction appeared. The response to the OCT was considered positive as a cutaneous and/or mucosal (erythema, wheals and/or angioedema) or respiratory (a decrease of at least 20% in the FEV1) manifestations appeared, or in case of hypotension. Emergency resuscitation equipment and personnel were available during the test along. No adverse reaction was observed. A week later another OCT with mannite was performed. Mannite is an oral laxative of 10 grams in weight (Mannite Dufour, Iuppa Industry, Alessandria, Italy) sold as OTC laxative. A galenic preparation was obtained by diluting 100 mg of mannite in 100 ml of sterile water. An initial dose of 1 mg/ml, and after one hour of 3 mg/ml were taken by the patient without any adverse reaction. Finally, 10 mg/ml were administered an hour later, but 45 minutes after this dose (total dose 14 mg/ml) the patient reported a generalized itching with an urticarial rash on the trunk, associated to lips angioedema without any drop in blood pressure. FEV1 decreased 15% from basal. The patient was immediately treated with intravenous methylprednisolone (40 mg) and chlorphenamine maleate (10 mg/ml) in 100 ml of saline, and the adverse reaction faded completely. The patient was addressed to the Laboratory of Allergy and Clinical Immunology Department in Bari University, to perform serum dosages of Olive recombinant allergens by Phadia-Thermo Scientific Inc. and a Basophil Activation Test, but he declined any further investigation. So, patient was correctly informed about his mannitol hypersensitivity and recommended to avoid mannitol present in drugs and in foods as an excipient, and to communicate his particular hypersensitivity in case of hospitalization.

Discussion

Preservatives, excipients and dyes in drug formulations represent a true puzzle for allergists and dermatologists. At a first sight, the active pharmaceutical molecule is usually considered the responsible agent for a hypersensitivity reaction following the drug assumption, but sometimes a more careful investigation is required for the correct identification of the culprit agent (3). Kaliskaner et al. described a 22 year old man treated with rifampicin for a tuberculosis lymphadenitis. After 11 months of treatment, the patient regularly developed skin eruptions showing as recurring, self-limited, macular, itchy rashes, symmetrically placed on the face, ears, buttocks, elbows and knees. The lesions appeared at the same time every

day and lasted about 45 min, then disappeared spontaneously without any treatment. After various investigations with oral challenge tests for each anti-tubercular drug assumed by patient, Authors identified the culprit agent in a blue dye, patent blue dye, present in a rifampicin branded formulation. Such dye was substituted by indigotin (indigo blue) in another branded rifampicin formulation which, on the contrary, was tolerated by the patient (2). The whole allergic work-up was rather elaborated and skin tests showed to be not very helpful to the patient in the diagnosis (2). In our case report, it was the assumption of the synthetic sweetener to alert about the necessity to perform a new allergic session, in order to investigate the patient more carefully. Mannitol is a white crystalline sugar also named *mannite* or *manna sugar*. Manna is one of most ancient sweeteners in Europe before the introduction of the sugar cane. Mannitol is an acyclic hexitol sugar derived from the reduction of D-mannose (an aldohexose), which is not metabolized and therefore is excreted unchanged in the urine (4). For its hyperosmotic and diuretic properties, mannitol has been used for prophylaxis against acute renal failure due to toxic causes and to reduce cerebrospinal or intraocular fluid pressure (4). Although not so frequently reported in literature, D-mannitol is known to cause immediate-type hypersensitivity reactions when given intravenously (5-8). Such manifestations are usually attributed to mannitol hyperosmolar properties, able to trigger a non-specific mast-cells or basophils degranulation (8). For that reason, usually this immediate type hypersensitivity reactions are reputed to be non immunologic (6,8). In our case, the patient had assumed mannitol orally, so a hypertonic effect causing a direct mast-cell degranulation seemed to be excluded. On the contrary, Venkatesh and Hegde have proposed D-mannitol can induce a true IgE-mediated reaction (9). In their experience, D-mannitol usually exists as a cyclic form . However, in an aqueous solution, a very small amount of the acyclic form exists. D-mannose acts as a prosensitizer, the Schiff base conjugates with amino groups of proteins, as confirmed by their studies *in vivo* and *in vitro*, acts as a sensitizer, and lastly D-mannitol acts as a non-sensitizing elicitor (9). Moreover, they demonstrated in a patient the presence of circulating mannitol-specific human IgE by enzyme-linked immunosorbent assay (ELISA), using a D-mannitol-protein conjugate as coating antigen, both with affinity-chromatographed serum from the sensitized subject (10), because mannitol-specific IgE could not be detected in the allergic subject serum, probably for the binding of the hydrophilic mannitol (or any other sugar alcohol) to the hydrophobic polystyrene surface of microtiter wells (10). The presence of mast cell-bound mannitol-specific IgE in the patient was shown by positive SPT using D-mannitol–protein conjugates (10). This could explain why SPT gave a negative response in our patient. Mannitol is widely used in food industry as a sweetener and a dietetic substance, because its uptake is independent of insulin (4); it is thus applicable in diabetic and dietetic food products. Mannitol is also widely utilized in pharmaceuticals as excipient namely E421, according to European directives about food excipients (11). Mannite is the unrefined form of mannitol sold as an over-the-counter oral laxative, packaged like a butter pat. Because of a possible anaphylactic reaction by administering mannitol intravenously (5-8), we preferred to perform an oral challenge test in our patient, considering the oral route less hazardous and more ethical. In that way, we could calculate the administered dose, by stopping the challenge test as soon as patient had shown any symptom of adverse reaction. Recently, Australian researchers reported a 39 year old woman who had 3 anaphylactic reactions following intravenous administration of paracetamol, although the patient tolerated oral paracetamol. Skin tests and Phadia ImmunoCap to 1-amino-1-deoxy-d-mannitol confirmed the responsible agent was mannitol contained in intravenous formulation of paracetamol (12). Mannitol is the most widely distributed sugar alcohol in nature, and it has been reported in more than 100 species of vascular plants of several families, including the Oleaceae (olive, privet, ash tree) and the Apiaceae (celery, carrot, parsley) (13). Moreover, it has caused anaphylactic reactions as food allergen contained in pomegranate (*Punica granata*) (14) and mushrooms (15), as confirmed by skin tests in both the reported clinical cases (14,15). Interestingly, mannite for commercial and pharmaceutical purposes is obtained and collected by *Fraxinus species* trees (ash tree), which belong to the *Oleaceae* family. The amount of mannitol varies during the different seasons in the trees of *Fraxinus* species, while it is constant and always stable in Olive trees during the whole year (16). Alternatively, various purification processes are requested to extract mannitol from Olive leaves and separate it from its stereoisomer, sorbitol (17). Because our patient was afflicted by an Olive tree pollen rhinoconjunctivitis, probably Olive tree pollen allergy should be considered an undervalued risk factor for mannitol hypersensitivity, even in the light of the increased attention given to carbohydrates as allergens (18). A further botanical study investigated the average annual concentrations of starch and soluble sugars, including mannitol, in Olive tree leaves, branches, bark and roots, but unfortunately, not in pollen (19). According to the literature reports, alimentary route seems the most likely pathway able to induce mannitol sensitization, but there is also the possibility that, in our patient, mannitol hypersensitivity had been induced through the inhalant pathway, so, beyond a food allergen, a drug allergen and an excipient allergen, mannitol might even be a respiratory allergen.

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Allergic contact dermatitis in child with odontoiatric face-mask

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Key words
Nichel sulphate; contact dermatitis; odontoiatric device

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We report the case of an interesting odontoiatric 12 years old patient with nickel sulphate and fragrance mix allergy.

He presented to our department for erythematous papular itchy lesions, localized in the perioral region, arisen one year after the application of a particular dental appliance: adjustable dynamic protraction facemask-Ormco-Sybron dental specialties (**figure 1A**, **1B**, **1C**), with progressive worsening of the clinical picture, despite topical application of corticosteroids.

We executed patch test with standard series SIDAPA, official standardized series of haptens approved by the Italian Society of Professional and Environmental Allergic Dermatology (acronym for Società Italiana di Dermatologia Allergologica Professionale e Ambientale) carried out with F.I.R.M.A. support.

We applied on the back of the patient (by a single operator, A. Tammaro) two patches containing the following haptens: Potassium Dichromate; Rosin; Epoxy Resin; Formaldehyde Resin; Euxil 400; Neomycin Sulphate; Fragrance Mix; Nickel Sulphate; Mercaptobenzothiazole Paraphenylendiamine; Cobalt Chloride; Balsam of Peru; Thiuram Mix; Benzocaine; Lanolin Alcohols; Parabens; Vaseline; Scattered Yellow; Scattered Blue; Hydroquinone (1).

The patient was asked to do not wash his back and do not take orally corticosteroids and antihistamines.

The patient came back after 48 hours at our clinic: the operator who applied the patches removed them, making the first reading.

The patient returned after 24 hours for the second reading at 72 hours.

The test is positive if the sites of contact with haptens show signs like erythema (+ positive), erythema and vesicles (+ + positive), erythema and vesicles and edema (+ + + positive).

The patch test applied on our patient resulted positive for nickel sulphate (++) and fragrance mix (+). Adjustable dynamic protraction facemask contains nickel sulphate.

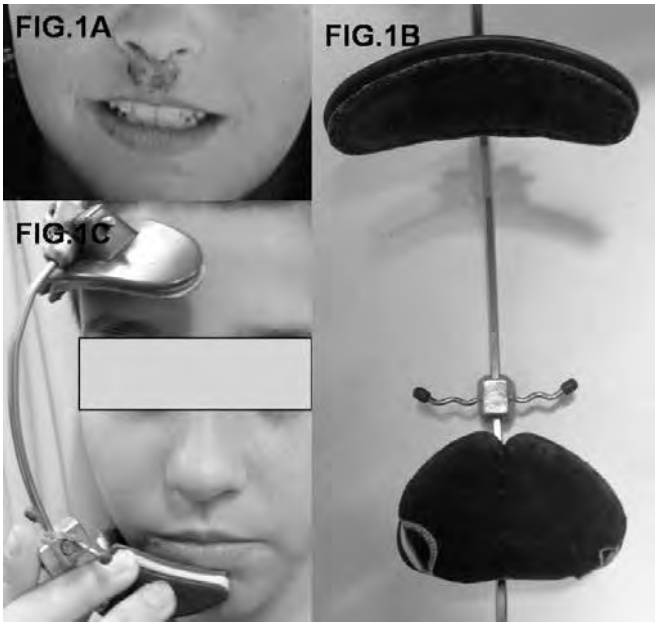
Dental correction by adjustable dynamic protraction facemask occurs by a combination of skeletal and dental changes in both sagittal and vertical dimensions. These changes occur as a result of forward movement of the maxilla, backward and downward rotation of the mandible and proclination of the maxillary incisors. Other odontoiatric-facial changes contributing to class III correction shown to occur with facemask and palatal expansion treatment are downward movement and counter-clockwise rotation of the maxilla, increased convexity in the middle face with forward displacement of orbital and key ridge, increase in maxillary depth and lower facial height, anterior movement of maxillary molars and incisors, decrease in SNB, as well as inferior movement of B-point, pogonion and menton. Soft-tissue changes contributing to increased convexity of the profile are anterior movement of pronasale, subnasale, and labrale superius, as well as inferior movement of the soft-tissue chin. When comparing the contribution of orthopedic and orthodontic effects with facemask and palatal expansion therapy, nearly all investigators attribute the majority of Class III correction to orthopedic movement, with most of the change taking place in the maxilla (2).

The gold standard of treatment consists in wearing the face mask for 18 hours/daily until the age of twelve. Our patient developed skin lesions about 2 years after the application of the device and, after the removal of facial mask, the skin lesions resolved.

Fragrance mix allergy was not related with dental device, in fact the patient showed erythematous papular lesions after the use of products containing fragrance. This data is not relevant to the clinical case reported. It is a clinical accidental data, that we can not actually explain.

We suggest it could be interesting to conduct further studies to investigate the development of allergy to fragrance mix in children, since they are a kind of population usually little exposed to contact with this allergen (3).

Figure 1A *- Erythematous papular itchy lesions, localized in the perioral region arisen one year after the application of the dental appliance shown in* **figure 1B** *and* **1C**.



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