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Uncovering new potential culprits in drug allergy: non-vitamin K oral anticoagulants

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New anticoagulant drugs (Non-vitamin K Oral AntiCoagulants, NOACs) have massively entered the pharmaceutical market and are increasingly being prescribed as an alternative to vitamin K antagonists in the prevention and treatment of thromboembolism and in the prevention of stroke in atrial fibrillation (1,2). Predominantly skin adverse reactions were mentioned as side effects since the first clinical trials and isolated case reports have recently shed light on the possible role of these drugs in the induction of a specific immune-mediated response (3-7).

The present issue of *European Annals of Allergy and Clinical Immunology* focuses on emerging drug hypersensitivity reactions to NOACs as a novel chapter of drug hypersensitivity reactions (DHR) and for which a correct diagnostic approach has to be proposed and shared by allergists.

The article from Cortellini et al. (8) addresses the problem of performing a correct diagnosis in delayed reactions, assuming that the pathological mechanism is mediated by T lymphocytes (type IV DHR). The Authors report a case of delayed skin hypersensitivity reaction to factor Xa inhibitor edoxaban, in which the diagnosis was confirmed by epicutaneous tests, starting from the identification of a non-irritant concentration for edoxaban and all other NOACs. They also provide evidences of a good accuracy of this type of *in vivo* test especially at a late reading. Furthermore they highlight the possibility of cross-reactivity between different NOACs and suggest that warfarin may be tolerated as an alternative drug.

This work has prompted a more extensive review of the literature in order to better understand and classify adverse drug reactions to NOACs and to identify the most common types of DHR.

Carli et al. (9) reviewed published reports of hypersensitivity reactions to these drugs, which show a predominance of delayed type III and IV reactions (both mild and severe), in particular for dabigatran and rivaroxaban, the earliest introduced drugs. Secondly, published papers confirm the previous suggestion by Cortellini et al. (8) that patients who reacted to NOACs, could afterwards tolerate warfarin and moreover that switching to low molecular weight heparins (LMWH) was found to be safe. A number of reported observations also leads to the hypothesis that rivaroxaban would not cross-react with other factor Xa inhibitors. The review (9) also stresses the importance of safety in dealing with a patient with a probable hypersensitivity reaction to a NOAC. As anticoagulation effect must be maintained, a multidisciplinary management in a hospital setting should be mandatory while performing diagnostic tests. Regarding the diagnostic work-up, the Authors (9) point at the unmet needs of both identifying standard techniques for prick and intradermal tests and adapting available *in vitro* tests (e.g. anti-drug antibodies, basophil activation test, lymphocyte transformation test) in relevant reactions. They also propose patch tests as first diagnostic step in mild/moderate delayed reactions, as previously described by Cortellini et al. (8), performing late readings and subsequently starting a very slow oral challenge with an alternative NOAC which resulted negative to patch tests.

Taken together the two papers on NOACs published in this issue of *European Annals of Allergy and Clinical Immunology* highlight the importance of raising clinicians' awareness on the risk of immune-mediated reactions to novel anticoagulant drugs, which might still be underestimated. Due to the complexity of dealing with patients often receiving multiple medications and suffering from cardiovascular diseases or prothrombotic conditions, a multidisciplinary approach is always recommended. Diagnostic strat-

egies are still at an early stage for this new chapter of drug allergy but a first tool for the evaluation of delayed reactions was provided: patch tests are easily available in the clinical practice. Nevertheless, more clinical and laboratory research is needed to go beyond the current probability scores and obtain a general consensus on standardized techniques in all types of DHR to novel anticoagulants.

References

1. Hankey G, Eikelboom JW. Dabigatran etexilate: a new oral thrombin inhibitor. Circulation. 2011;123:1436-1450.
2. Giugliano RP, Ruff CT, Braunwald E, et al. Edoxaban versus warfarin in patients with atrial fibrillation. N Engl J Med. 2013;369(22):2093-2104.
3. Altin C, Ozturkeri OA, Gezmis E, Askin U. Angioedema due to the new oral anticoagulant rivaroxaban. Ann Card Anaesth. 2014 Apr-Jun;17(2):173-4.
4. Hasbal NB, Baştürk T, Koç Y, Sahutoğlu T, Bayrakdar Çağlayan F, Ünsal A. Leukocytoclastic Vasculitis Associated with a New Anticoagulant: Rivaroxaban. Turk J Haematol. 2017 Mar 1;34(1):116-117
5. Radu C, Barnig C, de Blay F. Rivaroxaban-Induced Drug Reaction with Eosinophilia and Systemic Symptoms. J Investig Allergol Clin Immunol. 2016;26(2):124-6. 28.
6. Nasir UB, Kumar A, Easwar A. Apixaban causing Leukocytoclastic Vasculitis. J Allergy Clin Immunol Pract. 2018 Jan 19. pii: S2213-2198(17)31025-5.
7. An J, Garje R, Wanat KA, Leone JP. Dabigatran-related leukocytoclastic vasculitis. BMJ Case Rep. 2017 Jan 4;2017.
8. Cortellini G, Rossi F, Lippolis D, Cortellini F, Gavioli B, Ballardini G. Delayed hypersensitivity to new oral anticoagulants. Demonstration of cross reactivity for the drug category and definition of non-irritant concentrations for patch tests. Eur Ann Allergy Immunol. 2019; 51:42-44.
9. Carli G, Farsi A, Chiarini F, Lippolis D, Cortellini G. Hypersensitivity reactions to Non-vitamin K Oral Anticoagulants – a review of literature and diagnostic work-up proposal. Eur Ann Allergy Clin Immunol. 2019; 51: 7-14.

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Hypersensitivity reactions to non-vitamin K oral anticoagulants - a review of literature and diagnostic work-up proposal

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Key words

novel oral anticoagulants or non-vitamin K oral anticoagulants (NOACs); rivaroxaban; apixaban; edoxaban; dabigatran; hypersensitivity

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Summary

Non-vitamin K antagonist oral anticoagulants (NOACs) are increasingly being used in hospital and outpatient settings as safe alternatives to warfarin. Hypersensitivity reactions have been described for NOACs and can be classified according to Gell and Coombs. We reviewed case reports of possible drug hypersensitivity reactions, noticing a predominance of delayed reactions (both mild and severe) and the absence of cross-reactions to warfarin and low molecular weight heparins. International experience on diagnostic tests is lacking. The vast majority of authors refer to probability scores and rely on biopsy to classify vasculitis and rule out differential diagnoses. We propose to adapt available tests to confirm the patient's reactivity to new anticoagulants. Among in vivo tests, patch testing revealed promising in delayed reactions.

Introduction

Non-vitamin K antagonist oral anticoagulants or novel oral anticoagulants (NOACs) are increasingly being used in the prevention of stroke in atrial fibrillation (AF) and in the prevention and treatment of venous thromboembolism (VTE) (**table I**). NOACs include direct thrombin inhibitor dabigatran and factor Xa inhibitors apixaban, edoxaban and rivaroxaban (1). They are generally considered as a safe alternative to vitamin K antagonists, overcoming the need of closely INR (international normalized ratio) monitoring and the risk of drug-food and drug-drug interactions. In addition, they have the advantage of a fixed dose and a relatively quick onset of action. Since their introduction from the early phase III studies, increasing numbers of patients have been treated with novel oral anticoagulants, which are now exceeding those treated with warfarin (2). As with warfarin therapy, most drug-related side effects are type A reactions (3), which are predictable pharmacological effects, linked to the mechanism of action: in this case, anticoagulation and bleeding risk increase. Among type B adverse drug reactions, which are unpredictable (also called "bizarre") reactions, hypersensitivity reactions have been described in patients treated with NOACs.
Aim of this review is to collect data about novel anticoagulant hypersensitivity reports, to classify the reactions and to define a possible approach for their diagnostic management.

Materials and methods

A medline search with the terms "novel anticoagulants OR DTI OR NOAC OR apixaban OR edoxaban OR rivaroxaban OR dabigatran AND dermatitis OR hypersensitivity OR reaction OR allergy OR urticaria OR angioedema OR vasculitis OR rash OR exanthema" was performed, leading to 33 results (last search: July 2018). Among the results, 25 articles matched

Table I *- Non-vitamin K anticoagulants indications.*

	VTE prevention	VTE treatment	NVAF
rivaroxaban	x (in hip and knee replacement surgery)	x	x
apixaban	x (in hip and knee replacement surgery)	x	x
edoxaban	-	x	x
dabigatran	x (in hip and knee replacement surgery)	x	x

VTE, venous thromboembolism; NVAF, non valvular atrial fibrillation.

with the purpose of this study. A thorough evaluation of in-label safety data and of clinical trials published provided further information. We attempted to classify the hypersensitivity reactions according to the Gell and Coombs classification (4), taking into account the immunopathogenetic mechanism predominantly involved: type I reactions are immediate (occurring within the first hour) and IgE-mediated; type II reactions are antibody-dependent cytotoxicity reactions; type III reactions depend on immune complex formation and deposition; type IV reactions are delayed type cellular hypersensitivity reactions.

Results

Through the literature search mentioned above we were able to identify 29 case reports of possible hypersensitivity reactions to NOACs (**table II**). The culprit drugs were mostly rivaroxaban

Table II *- Summary of reported drug hypersensitivity reactions (DHR) to novel oral anticoagulants.*

	rivaroxaban	apixaban	edoxaban	dabigatran
type I DHR	1 (U/AE + bronchospasm) *(Altin et al, 2014)*	< 1% *(product label)*	< 0.01% *(product label)*	< 0.1% *(RE-LY study)*
type II DHR	2 (thrombocytopenia) *(Mima et al, 2014; Pop et al, 2018)*	-	-	-
type III DHR	5 (leukocytoclastic vasculitis) *(Sainz-Gaspar et al, 2018; Dean et al, 2017; Hasbal et al, 2017; Chaaya et al, 2016; ROCKET trial 2011)* 1 (serum sickness) *(Snyder et al, 2015)*	1 (IgA leukocytoclastic vasculitis) *(Nasir et al, 2018)*	-	3 (leukocytoclastic vasculitis) *(An et al, 2016; Potolidis et al, 2015; Cakmak et al, 2014)*
type IV DHR	4 (DRESS or HES) *(Prasannan et al, 2013; Chiasson et al, 2017; Radu et al, 2016; Barrett et al, 2015)* 2 (MPE) *(Rudd et al, 2018, Sasson et al, 2017)* 2 (toxic skin eruptions) *(ROCKET trial 2011)* 1 (SJS/GBFDE) *(Vernon et al, 2016)* 1 (AGEP-like) *(Yates et al, 2013)* 2 (erythema multiforme/exfoliative rash) *(ROCKET trial 2011)*	1 (psoriasiform exanthem) *(Veliyev et al, 2016)* 1 (eczematous dermatitis) *(Cortellini et al, 2018)*	2 (skin rash) *(Kuroda et al, 2013; Cortellini et al, 2018 in press)* 1-10% (rash) (product label)	4 (MPE) *(Winkle er al, 2012; To et al, 2013; Eid e al, 2011; Cucurull et al, 2010)* 1 (TEN) (Tsoumpris et el, 2013) 5.3% (skin disorders) *(post-marketing data)*

U, urticaria; AE, angioedema; DRESS, drug rash with eosinophilia and systemic symptoms; HES, hypereosinophilic syndrome; MPE, maculopapular exanthema; SJS, Stevens Johnson syndrome; GBFDE, generalized bullous fixed drug eruption; AGEP, acute generalized exanthematous pustolosis; TEN, toxic epidermal necrolysis.

(16 cases) and dabigatran (8 cases). Of note, these drugs were the first to be introduced on the market in 2008 (**table III**). As for rivaroxaban, 5 cases were also described in the ROCKET trial (5). After a further analysis of data provided, a case of neurologic adverse reaction to edoxaban (6) was considered an idiosyncratic reaction and therefore excluded from the total of hypersensitivity reactions collected.

Table III *- Novel oral anticoagulants.*

	drug name	approval in Europe
direct thrombin inhibitors	dabigatran	2008
factor Xa inhibitors	rivaroxaban	2008
	apixaban	2011
	edoxaban	2015

Hypersensitivity reactions to rivaroxaban

Rivaroxaban is a factor Xa inhibitor indicated for the prophylaxis of stroke and systemic embolism in nonvalvular AF, for the prophylaxis of deep vein thrombosis (DVT) in patients undergoing knee or hip replacement surgery, for the treatment of DVT and pulmonary embolism (PE) and for the secondary prophylaxis of DVT and/or PE (7). Prescribing information leaflet reports skin and subcutaneous tissue disorders (pruritus and blisters) as side effects described by more than 1% (2.1%, 1.4%, respectively) of 4487 rivaroxaban-treated patients in RECORD 1-3 studies. Pruritus was also recorded in EINSTEIN DVT and EINSTEIN PE studies in 2.2% of patients. Immune system disorders as hypersensitivity, anaphylactic reaction, anaphylactic shock, angioedema, Stevens Johnson syndrome and thrombocytopenia have also been identified during post-marketing experience, but an estimation of the incidence of these side effects lacks. In real-world prospective observational study XANTUS the authors do not mention hypersensitivity reactions as side effects (8). In the ROCKET trial comparing rivaroxaban to warfarin, 2 patients experienced toxic skin eruption, one patient suffered from cutaneous vasculitis, one patient developed erythema multiforme and one patient had an exfoliative rash following rivaroxaban therapy, 2 patients had anaphylactic reactions (the latter proved unrelated to the drug by the investigators) (5). In a comparative study of rivaroxaban and dabigatran in Poland (9), in the group of patients receiving rivaroxaban 25% experienced pruritus, 8.3% experienced rash. Although anaphylactic reactions and angioedema are reported as possible side effects, we were able to find only one case describing urticaria and angioedema in addition to bronchospasm (10) after the fourth dose of rivaroxaban. The clinical picture calls back to an IgE mechanism, but the reaction has occurred upon first known contact with this drug. A possible explanation for this event may be a cross-reaction with IgE antibodies generated by previous contact with apparently unrelated and up to now unidentified chemicals. The patient received antihistaminic drugs, methylprednisolone and oxygen treatment, but neither blood tests (in particular no tryptase) nor other diagnostic tests, nor rechallenge were performed. Two cases of possible drug induced thrombocytopenia were described, acute and delayed. Acute thrombocytopenia occurred 48 hours after first drug exposure, resolved after withdrawal and developed again on rechallenge (11). A possible antibody-dependent mechanism may be postulated (12), in which the drug, by binding reversibly to platelet membrane proteins, induces structural changes in the membrane proteins resulting in new antigen exposure. Delayed-onset thrombocytopenia was associated with purpuric lesions 4 months after starting rivaroxaban therapy, which gradually resolved six days after discontinuation (13). Of note, a 3-day course of high dose intravenous immunoglobulins probably contributed to rapid improvement of platelet count.

Rivaroxaban was also considered responsible of four cases of leukocytoclastic vasculitis (14,15,16,17) which developed respectively 4, 7, 10 and approximately 90 days after the start of anticoagulation. Common characteristics were the appearance of purpuric papules with diffuse distribution and in particular involving the limbs, symmetrically. On histopathologic evaluation infiltration was mainly consisting of neutrophils with erythrocyte extravasation and vessel wall fibrin deposition. In all cases vasculitic lesions disappeared approximately one week after stopping rivaroxaban. Two patients were prescribed systemic steroids, two patients did not receive any treatment.

Anticoagulation with rivaroxaban was also linked with serum sickness (18), characterized by fatigue, arthralgia, rash with wheals, generalized swelling, hypertransaminasemia and bilirubin elevation, fever (38.6 °C), leukocytosis, low C3 and C4, occurring 10 days after starting the drug and responsive to supportive treatment together with rivaroxaban withdrawal.

Among delayed type IV hypersensitivity reactions, most cases were mild to moderate, requiring steroids and supportive therapy, as well as culprit drug discontinuation.

Nevertheless, a case of fatal hypereosinophilic syndrome (19) was reported in a patient treated with rivaroxaban (the authors do not mention the duration of treatment), who presented with hypereosinophilia with eosinophilic lung disease, dyspnea, bleeding, transverse sinus thrombosis, cerebral infarcts with hemorrhages and subsequently coma, myocardial infarction leading to multi-organ failure and death. Neither rash nor fever were described, therefore a diagnosis of DRESS cannot be made.

A drug-induced hypersensitivity syndrome (20) not fullfilling criteria for DRESS was experienced by a patient one day after restarting rivaroxaban: the patient presented with mild pruritic papular rash, elevated transaminases, mild anemia, C reactive protein elevation and biopsy revealed acute spongiotic dermatitis with perivascular lymphocytes and eosinophilic infiltrates. No treatment was required and signs and symptoms disappeared 48 hours after discontinuing drug. The patient tolerated enoxaparin as switch-therapy.
A case of possible AGEP developed in a surgical patient on the second day of rivaroxaban treatment, consistent of a diffuse maculopapular itchy rash with pustolosis and peripheral blood neutrophilia and eosinophilia (21). Rivaroxaban was taken off and concomitant oral antihistamines and topical steroid treatment contributed to rapid resolution of symptoms. Switch to tinzaparin was tolerated.
Two definite diagnoses of drug rash with eosinophilia and systemic symptoms (DRESS) were made in relation to rivaroxaban intake: the first occurred 10 days after drug introduction (22), and resolved after stopping rivaroxaban, with supportive therapy and long tapering of oral steroids. The second case developed after 6 months of rivaroxaban treatment (23) with a particular liver involvement, responding to corticosteroids and drug withdrawal. The patient subsequently tolerated switch to warfarin.
Milder delayed hypersensitivity reactions included drug-induced rashes described as morbilliform eruption (24) or urticarial rash (25) without systemic symptoms and internal organs' involvement, fading after steroids and drug discontinuation. These reactions developed within the first week of treatment (day 2 and day 7, respectively). Patients could tolerate enoxaparin and apixaban as alternative drugs.
Rivaroxaban has also been implied as culprit drug in a hypersensitivity reaction characterized by an itchy rash, desquamating skin and blistering (26) after the first dose, accompanied by renal function impairment and inflammatory markers, resolving with hyperpigmentation after prompt rivaroxaban discontinuation, topical steroids and switch to enoxaparin. A diagnosis of generalised bullous fixed drug eruption (GBFDE) or an initial form of Steven Johnsons syndrome was postulated. Switch to enoxaparin was tolerated.

Hypersensitivity reactions to apixaban

Apixaban is a factor Xa inhibitor indicated for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation (NVAF), for the prophylaxis of DVT and PE in patients who have undergone hip or knee replacement surgery, for the treatment of DVT and PE, and for the reduction in the risk of recurrent DVT and PE following initial therapy (27). Worldwide experience with apixaban is relatively small in contrast to rivaroxaban. According to product informative documents, hypersensitivity reactions (skin rash, anaphylactic reactions, allergic edema, etc.) and syncope were reported in less than 1% of patients treated.
Our literature search included a case of reversible neurologic symptoms (6) after the first apixaban dose confirmed by re-challenge, in which the authors suggest a type I hypersensitivity reaction although the symptoms - dizziness, loss of balance, diplopia, confusion - were not consistent with a histamine- or eicosanoid-mediated mechanism, but rather with an idiosyncratic event. Furthermore, the patient was not clinically evaluated and symptoms were merely reported subjectively. The patient was successfully switched to rivaroxaban.
One case of leukocytoclastic vasculitis was described after 10 days of apixaban (28), appearing as an erythematous rash of lower limbs quickly evolving into purpuric itchy and burning rash. On biopsy evaluation IgA and C3 stained positive at a perivascular level; infiltration of neutrophils was described around and inside the superficial vascular plexus together with focal fibrinoid vessel wall necrosis and in association with erythrocyte extravasation. This clinical picture resolved after stopping apixaban and introducing oral steroids. Switch to rivaroxaban was tolerated.
Apixaban was also linked to the development of a palmoplantar psoriasiform eruption (29), three days after its start. The relationship between the thick, scaly, hyperkeratotic, erythematous, and desquamative plaques and the drug was supported by histopathological features of skin biopsy consistent with those of drug-related psoriasiform eruptions. The patient improved after drug withdrawal and topical steroid therapy.
Interestingly, a case of widespread eczematous dermatitis (30) developed 7 days after graded challenge with apixaban in a patient with delayed drug hypersensitivity reaction (DHR) to edoxaban, suggesting a possible cross-reactivity between Fxa inhibitors.

Hypersensitivity reactions to edoxaban

Edoxaban is a factor Xa inhibitor with approved indication for the prevention of stroke and systemic embolism in adult patients with NVAF with one or more risk factors according to CHADS2 score and for the treatment of DVT and PE as well as for the prevention of recurrent DVT and PE in adults (31). Product information label reports the results of safety evaluations in the ENGAGE AF-TIMI 48 and Hokusai VTE studies including 21105 patients exposed to edoxaban. Anaphylactic reactions and allergic edema are listed as rare (≥ 1/10,000 to < 1/1,000), hypersensitivity reactions and urticaria as uncommon (≥ 1/1,000 to < 1/100), while rash and pruritus affect a considerable proportion of patients (1-10%). No further information about the timing and characteristics of reactions is provided.
During post-marketing surveillance one patient experienced rash (32) with edoxaban in Japan but more details are lacking.

Recently, another delayed hypersensitivity skin reaction was described (30), occurring as a widespread erythematous rash ten days after the start of edoxaban therapy. Symptoms resolved after stopping edoxaban. The authors provide the first attempt to diagnostic evaluation of skin reactions to novel oral anticoagulants as other T cell-mediated drug reactions: skin tests with heparins were negative; a galenic preparation at 10% and 30% concentration in vaseline was used to perform patch test. Cross-reactivity between edoxaban and dabigatran was demonstrated by patch tests. Subsequent use of apixaban was unsuccessful too, due to the occurrence of a delayed eczematous rash but the patient tolerated warfarin.

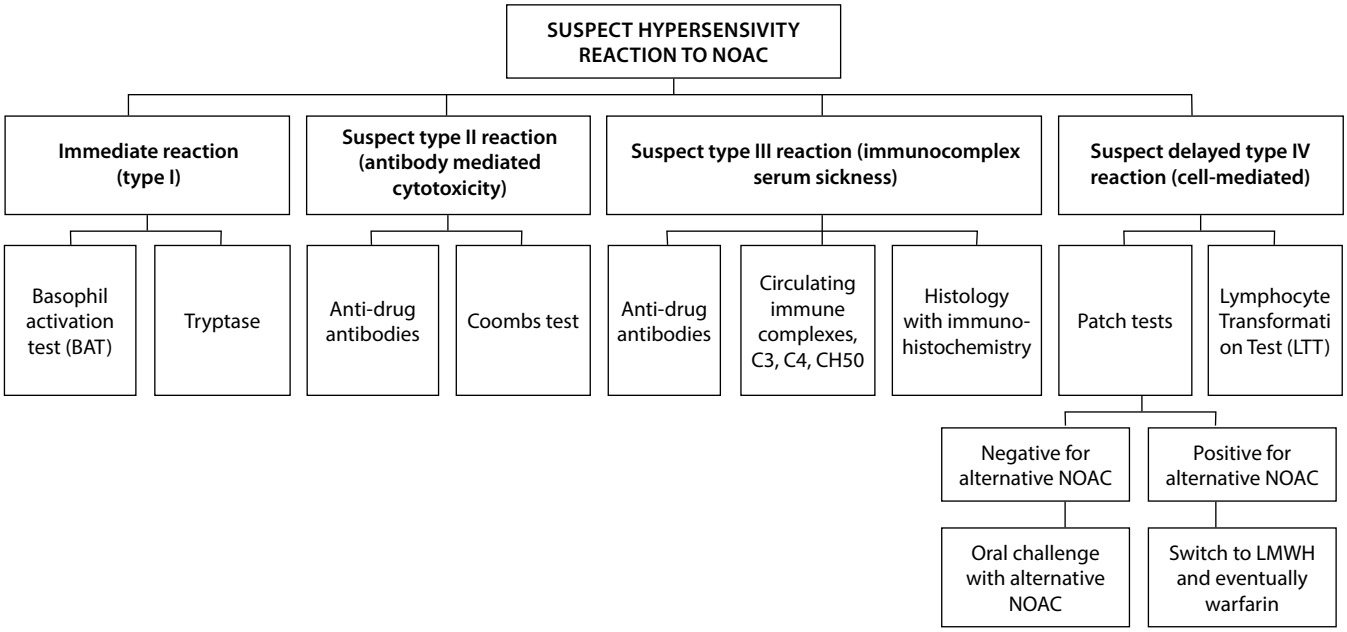
Hypersensitivity reactions to dabigatran

Dabigatran is a direct thrombin inhibitor indicated to prevent stroke and systemic embolism in patients with non-valvular atrial fibrillation, in the prophylaxis of VTE in patients who have undergone elective total hip or knee replacement surgery and in the treatment of DVT and PE and prevention of their recurrence (33,34). As reported in product information, in the RE-LY study drug hypersensitivity (including urticaria, rash, and pruritus), allergic edema and anaphylactic reactions occurred in less than 0.1% of patients. During post marketing safety surveillance skin and subcutaneous tissue disorders were notified with an incidence of 5.3% (35). A possible explanation for this relatively frequent side effect may be related to drug chemical structure as an aromatic amine (36).
Three leukocytoclastic vasculitis case reports were found (37,38,39) developing within the first week of treatment with dabigatran with typical purpuric macules, distribution to limbs, back and trunk, and shared histopathologic findings of neutrophilic infiltration, red cell extravasation and fibrin deposition within vessel walls. In the case described by An J. and colleagues (37) cutaneous vasculitis was associated with peripheral blood eosinophilia and elevation of inflammatory markers. Patients tolerated alternative anticoagulation (enoxaparin and warfarin) and resolution was generally rapid after dabigatran withdrawal and antinflammatory treatment (prednisolone, colchicine).
Four distinct cases of diffuse rash were clinically described as diffuse maculopapular rash (40), non pruritic maculopapular rash (41), diffuse, full-body pruritic rash (42) and urticarioid dermatitis (43). In the latter case, histologic data revealed an infiltration of lymphocytes, eosinophils with dermis vacuolization. The onset of symptoms ranged from 24 hours to 9 days after drug start. In all cases dabigatran discontinuation was sufficient for resolution.
The most severe reaction is a case of toxic epidermal necrolysis (45), in which dabigatran is one of the two possible culprits. The hypersensitivity reaction developed on restarting the drug after a brief discontinuation due to bleeding. Clinically, the patient presented with influenza-like symptoms and three days after erythematous symmetrical macules evolving into painful, burning vesicles and flaccid bullae with extensive sloughing, positive Nikolsky sign in approximately 70% of body surface (skin and conjunctiva). Treatment consisted of high dose intravenous immunoglobulins, antibiotics and wound care, together with culprit drugs interruption.

Discussion and proposal for a diagnostic work-up

Dealing with patients who presented a possible reaction to novel oral anticoagulants implies taking into account their need to be safely and rapidly anticoagulated and to monitor anticoagulation's side effects. In fact, although all NOACs have a predictable onset and offset of effect, not needing for routinal anticoagulation monitoring, kidney function should be assessed regularly to allow dose adaptation.
Data reported above highlight some common features of hypersensitivity reactions to Fxa inhibitors and DTI, particularly with respect to the majority of them, which belong to delayed type III and IV reactions. The possibility to switch patients to other anticoagulants is of utmost importance while performing the allergological diagnostic evaluation and the first evidence our search provides is that patients who reacted to NOACs, could afterwards tolerate warfarin and/or low molecular weight heparins (LMWH). The second observation arises from the case reports from Sasson et al. (25) and Cortellini et al. (30,unpublished), who stated that two patients reacting to edoxaban could tolerate rivaroxaban and that the patient with a previous reaction to rivaroxaban tolerated apixaban: rivaroxaban appears not to cross-react with other factor Xa inhibitors, whereas the same patient presented clinical and/or cutaneous reactivity to both factor Xa inhibitors (edoxaban, apixaban) and DTI (dabigatran).
We hence propose to manage a patient with a suspect reaction to NOAC as in **figure 1** in order to confirm diagnosis, according to time of onset and type of reaction, adapting available in vivo and/or in vitro tests, which are already being used in other drug allergies. After prompt discontinuation of the culprit drug, the patient should be switched to low molecular weight heparin to allow proper in-hospital drug hypersensitivity evaluation and diagnostic tests. The patient's indication for anticoagulation may then direct the choice and dose of alternative drugs.
We suggest using patch tests for the culprit drug and for other novel anticoagulants in the case of mild or moderate and delayed reactions. In vivo tests should be avoided in severe systemic reactions (i.e. SJS, TEN, HES), in which in vitro tests like lymphocyte transformation test (LTT) with the culprit drug may be experimentally performed in experienced laboratories. Patch tests should be prepared with whole tablets crushed in a mortar and mixed with vaseline at 30%. Readings are to be scheduled at 48 h, 72 h and 96 h. A graded challenge should be

Figure 1 *- Proposed diagnostic algorithm.*



performed with an alternative NOAC which resulted negative to patch tests. Due to the possibility of late reactions, we suggest a very slow challenge schedule (**table IV**), starting with a quarter of the total dose at day 1, then half dose at day 3 and a full dose from day 7 and subsequently daily, with concomitant close clinical and laboratory monitoring of side effects. Parallel heparin administration should be continued until 12 hours after reaching the full dose of NOAC.

Conclusions and research agenda

Novel oral anticoagulants appear to be most commonly responsible of delayed reactions, in particular type III and IV drug hypersensitivity reactions. Most cases of severe reactions are described for rivaroxaban, which has been used for longer. Although hypersensitivity was a relatively rare side effect, it is important to keep in mind the possibility, as well as for other anticoagulants, that NOACs may induce skin and also systemic reactions, particularly because they are being extensively and increasingly used as an alternative to warfarin. In addition, the lack of clinicians' awareness might underestimate the real incidence of hypersensitivity.
There is still scarce international experience on diagnostic tests to be performed in order to confirm the suspect of DHR to these novel drugs. The vast majority of authors refer to probability scores (e.g. Naranjo score, WHO-UMC Causality categories) to assess a correlation between the reaction and the use of the drug.

Table IV *- Challenge test proposed schedule.*

	day 1	***day 2***	***day 3***	***day 4***	***day 5***	***day 6***	***day 7***	***day 8 - 13***	***day 14***
graded challenge with NOAC (dose)	¼		½				1	1	1
LMWH anticoagulation							(last dose 12 h after reaching 1 dose NOAC)		
clinical evaluation	x	x	x	x	x	x	x		x
blood count	x		x		x		x		x
creatinine	x		x		x		x		x
PT, aPTT, fibrinogen	x		x		x		x		x

NOAC, novel oral anticoagulant; LMWH, low molecular weight heparin; PT, prothrombine; aPTT, activated partial thromboplastin time.

Biopsy is useful to confirm and classify the type of vasculitis. Other blood tests (e.g. serum autoantibodies, serology for infectious diseases...) are used to rule out other possible diagnoses. Taking into account the probable pathogenetic mechanism underlying the drug hypersensitivity reaction, we suggest to use available tests, in particular in vitro tests, to confirm the patient's reactivity to culprit drug. As for in vivo tests, there are no reports of attempts with skin prick tests or intradermal tests; patch testing revealed as a promising tool in one study instead. In fact, epicutaneous tests may be used in the future to evaluate alternative non-vitamin K antagonist oral anticoagulants and to guide subsequent oral drug tolerance test with a non-cross-reactive one in patients with mild delayed hypersensitivity reactions. Further studies might therefore confirm our preliminary observation of an absence of cross-reactivity between rivaroxaban and other NOACs.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Kirchhof P, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Heart J 2016; 37.
2. Van den Heuvel JM et al. NOACs Replace VKA as Preferred Oral Anticoagulant among New Patients: A Drug Utilization Study in 560 Pharmacies in The Netherlands. Thrombosis Journal 2018; 16:7.
3. Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosis, and management. Lancet 2000; 356:1255-1259.
4. Coombs PRGP. Classification of allergic reactions responsible for clinical hypersensitivity and disease. In: Gell RR, editor. Clinical Aspects of Immunology. Oxford: Oxford Univ Pr; 1968: 575-596.
5. Beasley N, Dunnmon P, Rose M. Clinical Review Application type: Standard, NDA 22-439 Xarelto (rivaroxaban). Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202439Orig1s000MedR.pdf
6. Josyln JA, Khattak FH, Geraci SA. A Case of a Reversible Neurologic Adverse Reaction to Apixaban Confirmed by Re-Challenge. J Clin Med Res 2018; 10(6):523-526.
7. Xarelto Package Insert. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/022406s015lbl.pdf (accessed: August 9, 2018)
8. Camm AJ et al. XANTUS: a real-world, prospective, observational study of patients treated with rivaroxaban for stroke prevention in atrial fibrillation. European Heart Journal 2016; 37(14):1145-1153.
9. Gorzelak-Pabiś P, Duraj I, Szlagowska L, Ciastkowska A, Broncel M. [Comparison of the safety of rivaroxaban versus dabigatran therapy in patients with persistent atrial fibrillation]. Pol Merkur Lekarski 2014; 37(221):261-264.
10. Altin C, Ozturkeri OA, Gezmis E, Askin U. Angioedema due to the new oral anticoagulant rivaroxaban. Ann Card Anaesth 2014; 17(2):173-174.
11. Mima Y, Sangatsuda Y, Yasaka M, Wakugawa Y, Nagata S, Okada Y. Acute Thrombocytopenia after Initiating Anticoagulation with Rivaroxaban. Internal Medicine 2014; 53(21):2523-2527.
12. Aster RH, Curtis BR, McFarland JG, Bougie DW. Drug-induced immune thrombocytopenia: pathogenesis, diagnosis, and management. J Thromb Haemost 2009; 7:911-918.
13. Pop MK, Farokhi F, Iduna L. Drug-induced thrombocytopenia after anticoagulation with rivaroxaban. Am J Emerg Med 2018; 36(3):531.e1-531.e2.
14. Dean R, Messer AM, Pickett M, Jahan-Tigh R. A case of leukocytoclastic vasculitis caused by novel anticoagulant rivaroxaban. Dermatol Online J 2017; 15:23(11).
15. Chaaya G, Jaller-Char J, Ghaffar E, Castiglioni A. Rivaroxaban-induced leukocytoclastic vasculitis: A challenging rash. Ann Allergy Asthma Immunol 2016; 116(6):577-8.
16. Hasbal NB, Baştürk T, Koç Y, Sahutoğlu T, Bayrakdar Çağlayan F, Ünsal A. Leukocytoclastic Vasculitis Associated with a New Anticoagulant: Rivaroxaban. Turk J Haematol 2017;34(1):116-117.
17. Sainz-Gaspar L, Pita da Veiga G, Suárez-Peñaranda JM, Vázquez-Veiga H, Sánchez-Aguilar D. Leukocytoclastic vasculitis associated with rivaroxaban. Int J Dermatol 2018; 57(5):622-624.
18. Snyder DJ, Matusik FB. Rivaroxaban-induced serum sickness after total knee arthroplasty. Am J Health Syst Pharm 2015; 72(18):1567-1571.
19. Prasannan N, Soultati A, Harrison C, Yeghen T, Radia D. 2 cases of idiopathic hypereosinophilic syndrome: a rare disorder with severe thrombohaemorrhagic complications [abstract]. Haematologica 2013; 98 Suppl1:627.
20. Chiasson CO, Canneva A, Roy FO, Doré M. Rivaroxaban-Induced Hypersensitivity Syndrome. Can J Hosp Pharm 2017; 70(4):301-304.
21. Yates J, Choudhry M, Keys G. A case report describing a suspected rivaroxaban hypersensitivity reaction in a surgical patient. J Clin Pharm Ther 2013; 38(2):159-161.
22. Radu C, Barnig C, de Blay F. Rivaroxaban-Induced Drug Reaction With Eosinophilia and Systemic Symptoms. J Investig Allergol Clin Immunol 2016; 26(2):124-6.
23. Barrett P, Vuppalanchi R, Masuoka H, Chalasani N. Severe drug-induced skin and liver injury from rivaroxaban. Dig Dis Sci 2015; 60: 1856-1858.
24. Rudd KM, Panneerselvam N, Patel A. Rash associated with rivaroxaban use. Am J Health Syst Pharm 2018; 75(6):347-349.
25. Sasson E, James M, Russell M, Todorov D, Cohen H. Probable Rivaroxaban-Induced Full Body Rash: A Case Report. J Pharm Pract. 2017:897190017722872.
26. Vernon HM, Nielsen AK, O'Bryan EC. Hypersensitivity reaction after administration of rivaroxaban (Xarelto). Am J Emerg Med 2016; 34(7):1325.e1-2.
27. Eliquis Package Insert. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/202155s000lbl.pdf (access: July 26, 2018)
28. Nasir UB, Kumar A, Easwar A. Apixaban Causing Leukocytoclastic Vasculitis. J Allergy Clin Immunol Pract 2018. pii: S2213-2198(17)31025-5.
29. Veliyev V. A rare side effect seen due to the use of apixaban: Palmoplantar psoriasiform drug eruption. Anatol J Cardiol 2016; 16:212-215.
30. Cortellini G, Rossi F, Lippolis D, Cortellini F, Gavioli B, Ballardini G. Delayed hypersensitivity to new oral anticoagulants. Demon-

stration of cross reactivity for the drug category and definition of non-irritant concentrations for patch tests. Eur Ann Allergy Clin Immunol 2018.
31. Lixiana Package Insert. Available at https://ec.europa.eu/health/documents/community-register/2015/20150619132091/anx_132091_en.pdf (accessed: September 2, 2018)
32. Kuroda Y, Hirayama C, Hotoda H, Nishikawa Y, Nishiwaki A. Postmarketing safety experience with edoxaban in Japan for thromboprophylaxis following major orthopedic surgery. Vasc Health Risk Manag 2013; 9(1):593-598.
33. Pradaxa Product Information FDA available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022512s007lbl.pdf (accessed: August 13, 2018)
34. Pradaxa Product Information EMA available at: https://www.ema.europa.eu/documents/product-information/pradaxa-epar-product-information_en.pdf (accessed: August 13, 2018)
35. Boehringer Ingelheim Dabigatran Briefing Document, 2010, available at https://www.researchgate.net/profile/Michael_Ezekowitz/publication/262733892_Stroke_prevention_in_atrial_fibrillation_established_oral_anticoagulants_versus_novel_anticoagulants-translating_clinical_trial_data_into_practice/links/576eea0808ae842225a8849c/Stroke-prevention-in-atrial-fibrillation-established-oral-anticoagulants-versus-novel-anticoagulants-translating-clinical-trial-data-into-practice.pdf (accessed: August 13, August)
36. Joint Task Force on Practice Parameters; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology; Joint Council of Allergy, Asthma and Immunology. Drug allergy: an updated practice parameter. Ann Allergy Asthma Immunol 2010; 105:259-273.
37. An J, Garje R, Wanat KA, Leone JP. Dabigatran-related leukocytoclastic vasculitis. BMJ Case Rep 2017; Jan 4; 2017.
38. Potolidis E, Mandros C, Kotsa K, Mitsiou E, Potolidis D, Fanourgiakis P. Dabigatran Associated Leukocytoclastic Vasculitis. Case Rep Med 2015; 2015:616109.
39. Cakmak MA, Sahin S, Cinar N, Karsidag S. Adverse skin reaction caused by dabigatran. Eur Rev Med Pharmacol Sci 2014; 18(18):2595.
40. Winkle et al. Dabigatran After AF Ablation Journal of Cardiovascular Electrophysiology Vol. 23, No. 3, March 2012.
41. To K, Reynolds C, Spinler SA. Rash associated with dabigatran etexilate. Pharmacotherapy 2013; 33(3):e23-7.
42. Eid TJ, Shah SA. Dabigatran-induced rash. Am J Health Syst Pharm 2011; 68(16):1489-1490.
43. Cucurull I, Canosa J, Vega-Molpeceres S, Just Sarobe M, Escoda García M. [Dabigatran induced exanthema]. Rev Clin Esp 2010; 210(11):590-591.
44. Tsoumpris A, Tzimas T, Gkabrelas K, Akritidis N. Iron complex, dabigatran and toxic epidermal necrolysis syndrome: a case-report. J Clin Pharm Ther 2013; 38(2):177-178.

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Oral allergy syndrome amongst young Mexicans: prevalence and associated factors

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Summary

Background. *Oral allergy syndrome (OAS) is the most common food allergy manifestation amongst adults. However, population studies aimed at estimating its prevalence and associated factors are scarce in Mexico. Objectives. To establish the prevalence of OAS in a sample of university students and to describe their clinical characteristics and its associated factors.* ***Methods.*** *From a sample group made up of 25,269 university students, the data corresponding to 1,200 students aged 18 to 25 was analyzed with a crosssectional approach. A structured questionnaire was used to identify OAS, its symptoms and related foods, and the personal history of atopic diseases. The associations between variables were calculated through logistical regression analysis.* ***Results.*** *The prevalence of OAS was 3.4%, with a 95% confidence interval (95% CI) of 2.5 to 4.6. The main oral symptoms reported were lip pruritus, edema and the sensation of pharyngeal oppression. Among the extra-oral complaints were: reddish coloration of the skin, body pruritus, abdominal pain, and abdominal bloating. The foods that were most frequently associated with OAS were fruits (68.5%), vegetables (22.0%) and seafood (19.3%). Through multivariate analyses, allergy to pollen and latex were found to be associated with OAS, OR 3.29; 95%: CI 1.53 to 7.10 and OR 5.53; 95% CI: 1.08 to 28.2, respectively.* ***Conclusions.*** *Notably, the prevalence of OAS varies according to the geographic area. Personal histories of allergy to pollen or latex were the main factors linked to OAS.*

Introduction

The term oral allergy syndrome (OAS), also known as pollen-food syndrome, describes allergic reactions that primarily manifest in the oral cavity, including pruritus and edema on the lips, tongue or palate, immediately after food intake (1). OAS is caused when allergens found in fruits, vegetables and pollen react in a sensitized subject (2). Although symptoms are usually limited to the mouth, some patients may also have extra-oral complaints, including pharyngeal edema, changes in skin color, or respiratory symptoms (3,4).

The prevalence of OAS is influenced by several factors; among them, the patterns of allergic sensitization in each geographic region and the prevalence of allergic rhinitis (2). In the non-selected population, the prevalence of OAS has been estimated between 2.0% and 11.5% (5-9).

Among the foods that have been most commonly linked to OAS are specimens from different botanical families: a) *Rosaceae* (pear, apple, peach, plum, strawberry, and almond); b) *Lauraceae* (avocado, walnut, and cinnamon); c) *Musaceae* (banana); d) *Bromeliaceae* (pineapple); e) *Actinidiaceae* (kiwi), and f) *Anacardiaceae* (mango) (4,10-14).

Additional factors linked to OAS prevalence are female sex (11,15), and allergic sensitization to pollens (11,16). The latter also influences the intensity of nasal and ocular symptoms (17). The lack of studies that determine the frequency of OAS among the young-adult population in Mexico motivates this study. The objectives of this study were to establish OAS

prevalence in a sample of university students and to describe the clinical characteristics and associated factors.

Methods

Design

The methods were previously published elsewhere (18). This was a cross-sectional study involving 25,269 students enrolled in the Autonomous University of the State of Mexico, with a final sample size of 1,200 students, aged 18 to 25 years, male and female, born in the State of Mexico, Mexico. The recruitment period took place from February to May 2014.

Questionnaire

Each participant filled out a questionnaire that helped us determine whether they were affected by OAS; it targeted demographic variables, their personal history of allergic diseases (asthma, allergic rhinitis, and atopic dermatitis), pollen or latex allergy, and the oral and extra-oral symptoms related to OAS, as well as the foods that caused it.

Definitions

To identify OAS, participants were questioned about the presence of oral symptoms (oral pruritus or lip edema) that had occurred immediately after consuming any food. Then, students reported the symptoms affecting other organs.

Ethics

The Ethics and Research Committee of the Center for Research in Medical Sciences of the Autonomous University of the State of Mexico approved our study (Registration No. 2014/05). To participate in the study, each student signed a consent form.

Statistical analysis

The prevalence of OAS was determined by calculating its frequency; additionally, its 95% confidence intervals (95% CI) were estimated. The association between the consumption of certain foods and the onset of OAS was also assessed. Furthermore, multivariate analyses were performed to identify factors associated with OAS. Statistical significance was estimated at $p \leq 0.05$. Data processing was performed with the IBM SPSS software, version 20.0 for Windows (Armonk, NY, USA).

Results

In total, there were 41 cases of OAS, with a prevalence of 3.4% (95% CI: 2.5 - 4.6); 3.2% amongst men and 3.6% amongst women (p = 0.719).
The sample consisted of students with a median age of 20 for females and 19 for males (p = 0.419) (**table I**). Among the subjects with OAS, 9.8% had asthma, 24.4% had allergic rhinitis and 14.6% had atopic dermatitis. There was no statistical difference between individuals of either sex, when it came to the personal history of asthma, allergic rhinitis, and

Table I *- Characteristics of the study group (n = 41).*

		Sex		
	total n = 41	**male n = 16**	**female n = 25**	**p**
Age (yr)				
median	19	19	20	0.419[1]
lower-upper limits	18 - 25	18 - 23	18 - 25	
Personal clinical history of allergic disease, n (%)				
asthma	4 (9.8)	2 (12.5)	2 (8.0)	0.637[2]
allergic rhinitis	10 (24.4)	3 (18.8)	7 (28.0)	0.712[2]
atopic dermatitis	6 (14.6)	2 (12.5)	4 (16.0)	0.999[2]
latex allergy	2 (4.9)	1 (6.3)	1 (4.0)	0.999[2]
pollen allergy	10 (24.4)	4 (25.0)	6 (24.0)	0.999[2]
Foods associated with OAS (n)				
median	2	2	2	0.606[1]
lower-upper limits	1 - 15	1 - 4	1 - 15	

[1] *p* value obtained by U de Mann-Whitney test, [2] *p* value obtained by Fisher exact test. $p \leq 0.05$ was considered statistically significant. OAS, oral allergy syndrome.

atopic dermatitis; as was the case for the allergy frequency to latex and pollen. The median number of foods associated with OAS was 2.

The three most important oral symptoms in subjects with OAS were lip pruritus, lip edema and sensation of pharyngeal oppression (**table II**). Almost half of the subjects that were studied had extra-oral symptoms; mostly cutaneous (reddening of the skin and itchy skin) and abdominal (abdominal pain and abdominal distention) symptoms. Respiratory symptoms were the least prevalent (cough, rhinorrhea or wheezing).

The foods most frequently associated with OAS included fruits (peach, kiwi and avocado), vegetables (chili, tomato, and bell pepper), seafood (shrimp), nuts (walnut and cashews), and legumes (beans and lentils) (**table III**).

Through multivariate analyses, the personal history of allergy to pollen and latex were identified as factors that are strongly

Table II *- Oral allergy syndrome clinical manifestations.*

	n = 41	%
Oral		
lip pruritus	37	90.2
lip edema	23	56.1
pharyngeal oppression	11	26.8
Extra-oral		
skin reddening	17	41.5
skin itching	14	34.1
abdominal pain	11	26.8
abdominal bloating	8	19.5
skin rash	7	17.1
lacrimation	6	14.6
heartburn	5	12.2
diarrhea	4	9.8
sneezing	4	9.8
dyspnea	4	9.8
sweating	4	9.8
flatulence	3	7.3
cough	3	7.3
rhinorrhea	3	7.3
wheezing	1	2.4

Table III *- Major foods related to oral allergy syndrome (n = 41).*

Food	n	%
Fruits	28	68.5
peach	8	19.5
kiwi	8	19.5
avocado	7	17.1
mango	5	12.2
apple	5	12.2
strawberry	3	7.3
pineapple	3	7.3
coconut	2	4.9
melon	2	4.9
guava	2	4.9
Vegetables	9	22.0
chili	4	9.8
tomato	3	7.3
bell pepper	1	2.4
cauliflower	1	2.4
mushrooms	1	2.4
Fish and shellfish	8	19.3
shrimp	7	17.1
octopus	2	4.9
Dairy products	7	17.1
milk	6	14.6
yogurt	4	9.8
cheese	1	2.4
Nuts and seeds	7	17.1
walnut	5	12.2
cashews	4	9.8
almond	3	7.3
hazelnut	3	7.3
brazilian nut	2	4.9
peanut	1	2.4
sesame	1	2.4
pistachio	1	2.4
Legumes	2	4.9
bean	1	2.4
lentil	1	2.4

associated with OAS, OR 3.29 (95% CI: 1.53 - 7.10 and OR 5.53 (95% CI: 1.08 - 28.2), respectively (**table IV**). Similarly, a personal history of atopic dermatitis was found to be closely related to OAS, OR 2.48 (95% CI: 0.98 - 6.28). The frequency of the latex-fruit syndrome was 2/41 (4.9%), one of the cases was related to kiwi and mango, and the other was linked to mango, melon, and papaya (**table IV**).

The pollen-food syndrome had a frequency of 10/41 (24.4%), where fruits were mainly involved; and women were more affected than men (ratio 1.5/1) (**table V**).

Table IV *- Multivariate models of factors associated with oral allergy syndrome in young adults.*

	Unadjusted model			Adjusted model		
	OR	95% CI	p	OR	95% CI	p
age	0.96	0.80 - 1.16	0.681	---	---	0.746
sex						
female	1					
male	1.02	0.53 - 1.97	0.941	---	---	0.901
asthma						
no	1					
yes	2.31	0.68 - 7.81	0.178	---	---	0.180
allergic rhinitis						
no	1					
yes	1.02	0.43 - 2.44	0.961	---	---	0.942
atopic dermatitis						
no	1			1		
yes	2.47	0.97 - 6.32	0.058	2.48	0.98 - 6.28	0.055
pollen allergy						
no	1			1		
yes	2.78	1.11 - 6.99	0.030	3.29	1.53 - 7.10	0.002
latex allergy						
no	1			1		
yes	5.53	1.05 - 29.23	0.044	5.53	1.08 - 28.2	0.040

Table V *- Food related to pollen-food syndrome.*

case	sex	age (yr)	foods
1	female	20	walnut, nutmeg
2	male	18	apple
3	female	19	banana, cauliflower, beans
4	female	20	coconut
5	female	22	kiwi
6	female	25	kiwi, mango
7	female	18	strawberry, kiwi, lime, lemon, orange, chili, tomato, pineapple
8	male	20	avocado
9	male	18	avocado, mango, walnut

Discussion

In this study, we observed that the prevalence of OAS amongst Mexican young adults from a public university was less than 5%, of whom a significant number showed extra-oral symptom in addition to oral discomfort, and fruits were found to cause OAS symptoms, in most cases. In addition, both pollen and latex allergy were highly associated with OAS.

Population-based studies aimed at determining the prevalence of OAS on a global level are scarce. Instead, most studies are based in clinics or hospitals and primarily involve patients suffering from allergic rhinitis, especially if they are sensitized to pollens (4,11,15). In our study, which was based on a young-adult population sample, the prevalence of OAS was 3.4%. Skypala et al. (5), also included an unselected population from the United Kingdom, with an OAS prevalence of 2%, which is very similar to ours.

In the state of Jalisco (in Western Mexico), a study of 18-50 year-old adults partaking physical activities during weekends, in public spaces, reported an OAS prevalence of 6.2% (8). In Northwest Portugal, a sample of adults from Porto reported a higher prevalence of OAS-related events with a frequency of 11.5% (9). In Eastern Europe, a study involving five countries (Sweden, Denmark, Estonia, Lithuania, and Russia) showed that the average prevalence of OAS in adults was 7.7% (6). Lastly, in Colombia, the frequency of pruritus and lip edema after consuming food was 4.7% (7). These differences in the prevalence of OAS might suggest that food availability, especially OAS-related foods, consumption habits, accompanied by sensitization to the pollens of a given region, act as triggers of OAS.

Approximately 30 years ago, Amlot et al. (19), described a group of 36 patients that reported oral discomfort after food consumption. Notably, half of them not only had symptoms confined to the mouth, but in addition, they had manifestations such as nausea, vomiting, abdominal pain, diarrhea, asthma, urticaria, and even anaphylaxis. In our study, the number of patients with extra-oral symptoms was consistent with the findings of this study. Amongst the most frequent symptoms found were skin problems, followed by gastrointestinal and respiratory problems. A study conducted in the United States by a group of allergists across the country, showed that up to 20% of patients with OAS and pollen allergy displayed extra-oral symptoms during food consumption (20). Another study, carried out in Mexican adult patients, who also had allergic rhinitis associated with pollen, showed extra-oral or systemic symptoms during fruit or vegetable intake with a rate of occurrence of almost 20% (4).

In Mexico, previous studies have documented that the main foods related to OAS are fruits and vegetables (4,10), which is consistent with reports in other parts of the world (11-14), being the most commonly associated factors with OAS the groups *Rosaceae* (peach, apple), *Lauraceae* (avocado, walnut), *Actinidiaceae* (kiwi), and *Anacardiaceae* (mango). However, among vegetables, chilies are more frequent in our study, likely because the Mexican diet includes them.

Interestingly, non-plant foods such as shellfish, milk and dairy products are also linked to OAS. It is likely that the initial symptoms are limited to the mouth, and then extra-oral or systemic manifestations are secondary features. Thus, it seems that there are two OAS phenotypes. The first one is limited to oral symptoms, mainly related to pollen sensitization and usually triggered by fruits and vegetables. The second phenotype not only expresses oral symptoms, but also includes various discomforts in other organs and systems that are similarly triggered by plant-origin foods; however, shellfish or dairy products can cause these as well. This group does not seem to meet the criteria for an anaphylactic reaction. In a conventional manner, they could be classified as OAS type I and OAS type II, respectively. More research is needed to clarify this point.

According to multivariate analyses performed in our study, sex was not related to OAS, and this same finding was observed in the Italian population (16). However, some studies report that sex is likely one main factor associated with OAS, since it is more prevalent in women (11,15). However, given the design of the studies with this conclusion, perhaps this difference does exist in a clinical setting, and this could be because women usually decide to go for medical care or perhaps the severity of their symptoms tends to be higher compared to men.

The factor most closely related to the onset of OAS is the sensitization to pollen grains from various plant species. In our study, the history of pollen allergy emerged as an element associated with OAS. However, we were unable to determine what type of pollen was involved. For example, birch pollen causes sensitization in subjects from large areas of Europe (16) and Japan (11), and more than 60% of patients that are sensitized to it, also manifest OAS. In our country, more than 50% of patients that were sensitized to pollen from the family *Oleaceae* (*Fraxinus*, *Ligustrum*, *Osmanthus*) express OAS (21).

Lastly, our study shows that a personal history of latex allergy is related to OAS. This is an unexpected finding since there were only two cases of latex-fruit syndrome. It is probably because in our study the foods most frequently associated with OAS were fruits, which have been widely known to share proteins that cross-react with latex (22,23). On the other hand, a cross-sectional study in patients with seasonal allergic rhinitis conducted in Split-Dalmatia indicated that the risk factors for OAS development were diabetes ($p < 0.001$), severity of nasal symptoms ($p < 0.05$), and severity of ocular symptoms ($p < 0.001$). However, due to the characteristics of our study we were not able to confirm this finding (17).

Remarkably, our study also allowed us to estimate the frequency of pollen-food syndrome; in the total population analyzed (1,200 subjects), ten subjects had symptoms compatible with it, for a prevalence of 0.8%. In this respect, to define the diagnosis of pollen-food syndrome, the students in our sample were

questioned if they had been diagnosed with pollen allergy by a physician (data not shown). One case caught our attention, as it was a female who not only had allergy symptoms produced by plant-derived foods, but also by animal-derived foods (milk, cheese, yogurt, clam, shrimp, crab, lobster, oyster, fish, and octopus). If this fact was due to simultaneous presence of OAS and pollen allergy then it should be considered that there are more people with these two conditions. To our knowledge, this is the first time that pollen-food prevalence is reported in a young-adult population, in Mexico and Latin America.

The main limitation of this study is that it relies on the self-reported assessment from each patient, as we did not conduct OAS tests during a medical interview or an oral challenge test. Thus, there is difficulty in interpreting the results. There was no way to verify the sensitization to pollen and latex during the time of our study; in a general manner of speaking, this is the nature of cross-sectional studies that are based on the use of questionnaires. It is also recommended to interpret the results according to the feeding habits and vegetation characteristics of each geographic area, as these have a remarkable influence on the frequency of OAS.

In conclusion, the frequency of oral allergy symptoms amongst young adults in our country differs from reported findings in other parts of the world, as it appears to be less frequent in the State of Mexico. Even within regions of Mexico, differences were found in the States of Jalisco (Western) and Mexico (Center). It is expected to observe that allergy to pollen and latex emerged as two relevant factors associated with OAS. Population-based studies are needed to establish the prevalence of OAS.

Additionally, we would like to highlight the necessity to classify the OAS in two phenotypes observed in our population. OAS type I that is limited to oral symptoms and OAS type II, which expresses oral and extra-oral symptoms.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Kondo Y, Urisu A. Oral allergy syndrome. Allergol Int 2009; 58:485-491.
2. Katelaris CH. Food allergy and oral allergy or pollen-food syndrome. Curr Opin Allergy Clin Immunol 2010; 10:246-251.
3. Mari A, Ballmer-Weber BK, Vieths S. The oral allergy syndrome: improved diagnostic and treatment methods. Curr Opin Allergy Clin Immunol 2005; 5:267-273.
4. Bedolla-Barajas M, Morales-Romero J, Ortiz-Miramontes LR, et al. Frequency and clinical features of the oral allergy syndrome in Mexican adults with nasal pollinosis. Rev Alerg Mex 2013; 60:17-25.
5. Skypala IJ, Bull SC, Deegan K, et al. Prevalence of pollen-related Oral Allergy Syndrome (OAS) in a UK population. J Allergy Clin Immunol 2010; 125:AB218-AB218.
6. Eriksson NE, Möller C, Werner S, Magnusson J, Bengtsson U, Zolubas M. Self-reported food hypersensitivity in Sweden, Denmark, Estonia, Lithuania, and Russia. J Investig Allergol Clin Immunol 2004; 14:70-79.
7. Marrugo J, Hernández L, Villalba V. Prevalence of self-reported food allergy in Cartagena (Colombia) population. Allergol Immunopathol (Madr) 2008; 36:320-4.
8. Bedolla-Barajas M, Bedolla-Pulido TR, Camacho-Peña AS, González-García E, Morales-Romero J. Food hypersensitivity in Mexican adults at 18 to 50 years of age: a questionnaire survey. Allergy Asthma Immunol Res 2014; 6:511-516.
9. Falcão H, Lunet N, Lopes C, Barros H. Food hypersensitivity in Portuguese adults. Eur J Clin Nutr 2004; 58:1621-1625.
10. Rodríguez-Mireles KA, Gaspar-López A, López-Rocha EG, Del Rivero-Hernández L, Segura-Méndez NH. Oral allergy syndrome in adults of a third level hospital. Rev Alerg Mex 2014; 61:65-72. In Spanish.
11. Gotoda H, Maguchi S, Kawahara H, Terayama Y, Fukuda S. Springtime pollinosis and oral allergy syndrome in Sapporo. Auris Nasus Larynx 2001; 28 Suppl:S49-52.
12. Cuesta-Herranz J, Lázaro M, Figueredo E, Igea JM, Umpiérrez A, De-Las-Heras M. Allergy to plant-derived fresh foods in a birch- and ragweed-free area. Clin Exp Allergy 2000; 30:1411-1416.
13. Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. Clin Exp Allergy 1994; 24:367-374.
14. Inomata N, Morita A, Kirino M, et al. Oral allergy syndrome due to plant-derived foods: a clinical review of 63 patients over a period of 6 years. Arerugi 2007; 56:1276-1284. (Abstract. In Japanese).
15. Caliskaner Z, Naiboglu B, Kutlu A, et al. Risk factors for oral allergy syndrome in patients with seasonal allergic rhinitis. Med Oral Patol Oral Cir Bucal 2011; 16:e312-6.
16. Asero R, Massironi F, Velati C. Detection of prognostic factors for oral allergy syndrome in patients with birch pollen hypersensitivity. J Allergy Clin Immunol 1996; 97:611-616.
17. Ivičević N, Roje Ž, Kljajić Z, et al. Prevalence and risk factors for developing oral allergy syndrome in adult patients with seasonal allergic rhinitis. Acta Clin Croat 2015; 54:25-29.
18. Puente-Fernández C, Maya-Hernández RL, Flores-Merino MV, et al. Self-reported prevalence and risk factors associated with food hypersensitivity in Mexican young adults. Ann Allergy Asthma Immunol 2016; 116:523-527.
19. Amlot PL, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS): symptoms of IgE-mediated hypersensitivity to foods. Clin Allergy 1987; 17:33-42.
20. Ma S, Sicherer SH, Nowak-Wegrzyn A. A survey on the management of pollen-food allergy syndrome in allergy practices. J Allergy Clin Immunol 2003; 112:784-788.
21. Morfín-Maciel BM, Flores I, Rosas-Alvarado A, Bautista M, López-López JR. Sensibilización a pólenes de la familia Oleaceae en un grupo de pacientes de la Ciudad de México. Rev Alerg Mex 2009; 56:198-203. In Spanish.
22. Blanco C. Síndrome látex-frutas. Allergol et Immunopathol 2002; 30:156-163.
23. Blanco C. Latex-fruit syndrome. Cur Allergy Asthma Rep 2003; 3: 47-53.

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Bet v 1 sensitization modulates allergenic molecular immune response

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Summary
Background. *Allergy is characterized by allergen-specific IgE production. Molecular-based allergy diagnostic allows to define the precise sensitization profile. Bet v 1 is the major allergen of the PR-10 family. It has been reported that pan-allergens could affect the sensitization panel in adults. This study aimed to evaluate the impact of Bet v 1 sensitization on sensitization pattern in a large sample of children.* ***Methods.*** *Serum IgE molecular components were assessed by ISAC method. Sera from 1,205 children, 708 males (58.76%) and 497 females (41.24%), median age 8.61 years (4.93 - 12.54 years) were analyzed.* ***Results.*** *A total of 354 PR-10-positive subjects were detected out of 1,205 subjects. Bet v 1 positive children were significantly more frequently sensitized to other molecules belonging to PR-10 family and noteworthy also to other allergenic families than Bet v 1 negative children.* ***Conclusions.*** *The present study demonstrates that Bet v 1 sensitization may significantly affect the sensitization pattern in children living in Genoa, a Mediterranean city located in a birch-free area.*

Introduction

Allergen-specific IgE production, such as sensitization, is the main biomarker of allergy. The natural history of allergy is usually characterized by a progressive increase of the number of sensitizations: the so-called poly-sensitization (1,2). From a clinical point of view, poly-sensitization is relevant, as its prevalence may be up to 90% of allergic subjects (3,4). The work-up in poly-sensitized patients, mainly concerning allergen immunotherapy prescription, has considerably advanced since the introduction of molecular-based allergy diagnostic, that is built on the assessment of allergen molecules (5,6). This methodology allows to precisely define and exactly characterize the sensitization profile by detecting the major allergens and excluding false reactivity to pan-allergens. Pan-allergen is an allergen molecule shared by different allergen sources. The main pan-allergens involved in pollen allergy are: PR-10, profilin, and LTP (7-9). PR-10 (pathogenesis-related protein group 10) molecules are appointed to defend plants against harmful microorganisms. PR-10 proteins were initially detected in pollens of *Fagales* order, mainly concerning birch, and further in cross-reacting fruits and vegetables (10). In the context of PR-10 family, the major allergen is Bet v 1, mainly contained in the pollens of the European white birch (*Betula verrucosa*) and in other trees of the *Betulaceae* family, including alders, hazels, hornbeams, hazel-hornbeam, and hop-hornbeams (11).

In our geographic area, *Betulaceae* allergy (BA) is very common: about 50% of patients with respiratory allergy are sensitized to *Betulaceae* pollens (12). However, this area is curiously birch-free: other pollen allergens related to PR-10 act as sensitizing primer, i.e. hazelnut and hornbeam. Previously, it has been demonstrated that serum IgE to Bet v 1 measurement may be useful to discriminate mere sensitization from true allergy in clinical practice (13). Recently, it has been reported that Bet v

1 sensitization is frequently associated with well-defined co-sensitizations and a peculiar sensitization pattern in adult subjects (14). Therefore, the present study aimed at testing the hypothesis that sensitization to Bet v 1 could affect the sensitization pattern also in children.

Material and methods

Patients

This retrospective study considered subjects suffering from respiratory complaints suggestive for allergy, as previously tested by skin prick test and/or serum IgE measurement, for allergen extracts, with positive findings. They went to the Laboratory of the Istituto Giannina Gaslini in Genoa (Italy) for serologic molecular assessment between July 2012 and April 2014. We analyzed the findings of serum allergen-specific IgEassessed by the ISAC method. The Review Board of the Istituto Giannina Gaslini approved the procedure. The patients' parents gave a written informed consent.

IgEAssay

Serum IgE were measured by ISAC test according to the manufacturer's recommendations (Thermo-Fisher Italy, Milan, Italy). Synthetically, 20 µL of the patient's serum were incubated on the microchip containing 112 allergen spots. After 1-hour incubation, slides were washed and a monoclonal anti-IgE antiserum labeled with a fluorochrome was added and incubated for 1 hour. Then, slides were re-washed and the chips were analyzed by a Laser Scan Confocal microarray reader (LuxScan 10K/A, CapitalBio, Beijing, China). A microarray Image Analyser immediately analyzed the findings. All samples were identified using a single barcode. The results were calculated by the software. The ISAC score was considered as ISAC Standardized Units (ISU), ranging from 0 to 100. Positive finding, such as sensitization, was defined by a value > 0.3 ISU, according to the manufacturer's rules.

Data and statistical analysis

Within each group (i.e. Bet v 1 positive and Bet v 1 negative), the number of positive tests, expressed as percentage, was evaluated. IgE levels were non-normally distributed and were summarized as medians with lower and upper quartiles. The Shapiro-Wilk test was used to evaluate the normality of the distributions. Frequency of positivity towards each allergenic molecule in Bet v 1 positive and Bet v 1 negative groups was compared by the Chi-square or Fisher's Exact test (as appropriate). IgE levels were compared using the Mann U Whitney test.
All the tests were two-sided and a p value < 0.05 was considered as statistically significant.

To identify and graphically display patterns of positivity to allergenic molecules sensitization, multiple correspondence analysis (MCA) (15) was performed. This method is a multivariate descriptive technique used for modeling a set of categorical variables modalities (positivity or negativity to a specific allergenic molecule) as points on a plane. The entire data set comprised Bet v 1, Act d 8, Aln g 1, Api g 1, Ara h 8, Cor a 1.01, Cor a 1.04, Gly m 4, Mal d 1, and Pru p 1.
To perform this analysis, a disjunctive table (Burt table) has been constructed (table not shown); in this table absolute frequency of the subjects presenting each modality of the variables (positive vs negative) either for each singular allergenic molecule (diagonal elements) (ex: number of subjects Bet v 1 positive) or combination of pairs of different allergenic molecules (ex: number of subjects Bet v 1 positive and Mal d 1 positive or number of subjects Bet v 1 positive and Mal d 1 negative, etc.).
The MCA technique converts this data set into a particular type of a 2-dimensional graphical display known as "factor plane" in which the 2 generated axes are the ones that explain the majority of variability ("inertia"). Therefore, the two axis are generated on the basis of the relationships between the variables included in the specific data set, and the first one (F1) is the Factor that explains most of the variability and the second one (F2) is the second important Factor. In the "factor plane", points represent different modalities of the sensitization variables and they are closely displayed when they share similar profiles with a subset of other sensitization variables and are displayed as distant when they are mostly dissimilar. Only the most representative factor plane (F1 and F2) according to the total inertia explained is presented in the following analysis.
Statistica software 9.0 (StatSoft Corp., Tulsa, OK, USA) was used for all the analyses and XLStat 2.0.1 (Addinsoft Co., New-York, USA) for the MCA.

Results

Sera from 1,205 patients, 708 males (58.76%) and 497 females (41.24%), median age 8.61 years (4.93 - 12.54 years) and age range 0 - 17 years, were analyzed. A total of 354 PR-10-positive allergic individuals [221 males (62.5%) and 133 females (37.5%), median age 10.47 (7.59 - 13.97) years] were detected out of 1,205 subjects. The analysis included all subjects: they were subdivided in two sub-groups: Bet v 1 positive and Bet v 1 negative. Bet v 1 represented the most commonly recognized PR-10, being 340 out of 354 (96.04%) PR-10-positive participants, followed by Cor a 1.01 (292; 82.49%).
Concerning possible gender-related difference, no significant difference was found in molecule recognition profile (p = 0.27). In addition, the age did not significantly impact on molecular spreading, i.e. the number of sensitizations (p = 0.33): the median age in mono-sensitized subjects was 9.54 (5.53 - 13.8)

years, whereas in poly-sensitized ones was 10.54 (7.84 - 13.97). Mal d 1, Cor a 1.04, Aln g 1, and Pru p 1 were positive in more than half of the patients.

Comparison between Bet v 1 positive and Bet v 1 negative subjects

Table I shows the frequency of positive tests to PR-10s in the whole population, and in Bet v 1 positive and Bet v 1 negative. We found that the frequency of sensitization to the other 9 PR-10s was significantly higher in Bet v 1 positive patients than in Bet v 1 negative patients (**table I**). The vast majority of Bet v1 positive subjects was also sensitized to other allergenic molecules (i.e, other than PR-10s), whereas two-third of Bet v 1 negative subjects had positive tests to other allergenic molecules (99.4% and 66.5%, respectively, $p < 0.0001$) (**table I**). Of 865 patients negative for Bet v 1, only 14 (1.6%) subjects were positive to the other 9 PR-10s, and particularly to Cor a 1.01 and Cor a 1.04 (**table I**).

Analyzing only sensitization to PR-10s, 40 out of 354 (11.3%) reacted to only one of the 10 PR-10s studied, of which 27 (67.5%) reacted only to Bet v 1. The second most frequent mono-reactivity was to Cor a 1.01 (no. 4; 10%) and to Cor a 1.04 (no. 4; 10%). In Bet v 1 positive group, sensitization to different pollen allergenic molecules such as Cor a 1.01 Aln g 1 (PR-10 proteins), nOle e 1, rPar j 2 and rPhl p 1 (**table II**) or to few plant food allergenic molecules such as rMal d 1, rCor a 1.04, rPru p 1 (all PR-10 molecules) (**table III**) was found in at least 50% of the studied subjects. In Bet v 1 negative group, only a limited number of subjects was sensitized to pollen (**table II**) or to plant food (**table III**) allergenic molecules, being the frequency of positive tests always below 20%. The most common profilin in our sample was rMer a 1.

As compared to Bet v 1 negative group, Bet v 1 positive subjects showed significantly more frequently high (i.e. > 15 ISU) or moderate (i.e. between 1 and 15 ISU) levels of IgE towards rCor a1.01, rPar j 2, rPhl p 5b, nAmb a 1, nCup a 1, nOle e 1, rMal d 1, nAct d 1 and nJug r 3 ($p < 0.05$, each comparison). A high proportion of Bet v 1 positive subjects also showed high or moderate levels of IgE towards some pollen (nAmb a 1) or plant food (rGly m 4, rAra h 8, nAct d 8) allergenic molecules that was totally absent in Bet v 1 negative group.

Median levels of IgE towards rPar j 2, nAmb a 1, nOle e 1, rCor a 1.01, nCup a 1, nAra h 3 (**table IV**), rMal d 1, nAct d 2, nJug r 3 (**table V**) were significantly higher in Bet v 1 positive than in Bet v 1 negative subjects with the only exception of rMer a 1.

Multiple correspondence analysis was performed to evaluate the possible mutual interrelationships among different PR-10s on the basis of IgE reactivity. Since this technique places variables based on their levels of reciprocal relationship (i.e., highly related variables are close to each other, while unrelated variables are placed far away from each other), we found that Bet v 1 positivity is frequently associated to sensitization to all the other pollen-derived molecules (i.e., Cor a 1.01, Aln g 1) and to some plant-food allergens (i.e. Mal d 1, Pru p 1 and Cor a 1.04). A

Table I *- Demographic and allergic sensitization characteristics of Bet v1 1-positive and Bet v 1-negative children.*

	Bet v 1 pos (no. 340)	**Bet v 1 neg (no. 865)**	**p value**
age (yrs) [median (LQ-UQ)]	10.6 (7.7 - 14.0)	7.5 (4.0 - 11.9)	**< 0.001**
gender [no. (% male)]	210 (61.8%)	498 (57.6%)	0.18
sensitization to other PR-10 proteins [no. (%)]	313 (92.1%)	14 (1.6%)	**< 0.0001**
rCor a1.01, protein PR-10	287 (84.4%)	5 (0.6%)	**< 0.001**
rMal d 1, protein PR-10	276 (81.2%)	3 (0.3%)	**< 0.001**
rAln g 1, protein PR-10	248 (72.9%)	0	**< 0.001**
rCor a1.04, protein PR-10	246 (72.4%)	4 (0.5%)	**< 0.001**
rPru p 1, protein PR-10	237 (69.7%)	2 (0.2%)	**< 0.001**
rGly m 4, protein PR-10	127 (37.4%)	0	**< 0.001**
rAra h 8, protein PR-10	126 (37.1%)	0	**< 0.001**
rApi g 1, protein PR-10	87 (25.6%)	2 (0.2%)	**< 0.001**
nAct d 8, protein PR-10	42 (12.4%)	0.0	**< 0.001**
sensitization to other allergenic molecules (other than PR-10 proteins) [no. (%)]	338 (99.4%)	575 (66.5%)	< 0.0001

Table II *- Frequency of positive test to different pollen allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Frequency [N (%)] of positive test among		
Allergenic molecules	**Bet v 1 pos children**	**Bet v 1 neg children**	**P**
rCor a1.01, PR-10 protein	287 (84.4%)	5 (0.6%)	**< 0.001**
rAln g 1, PR-10 protein	248 (72.9%)	0 (0%)	**< 0.001**
nOle e 1, group 1 *Oleaceae*	215 (63.2%)	139 (16.1%)	**< 0.001**
rPar j 2, LTP	173 (50.9%)	98 (11.3%)	**< 0.001**
rPhl p 1, group 1 *Graminae*	170 (50%)	131 (15.1%)	**< 0.001**
nCup a 1, pectate lyase	157 (46.2%)	94 (10.9%)	**< 0.001**
nCyn d 1, group 1 *Graminae*	152 (44.7%)	100 (11.6%)	**< 0.001**
nCry j 1, pectate lyase	116 (34.1%)	49 (5.7%)	**< 0.001**
nPhl p 4, berberine bridge enzyme-like protein	86 (25.3%)	55 (6.4%)	**< 0.001**
rPhl p5b, group 5 *Graminae*	73 (21.5%)	45 (5.2%)	**< 0.001**
rPla a 3, LTP	63 (18.5%)	50 (5.8%)	**< 0.001**
nPla a 2, polygalacturonase	61 (17.9%)	28 (3.2%)	**< 0.001**
rMer a 1, profilin	59 (17.4%)	18 (2.1%)	**< 0.001**
nArt v 3, LTP	53 (15.6%)	48 (5.5%)	**< 0.001**
rPhl p 2, group 2 *Graminae*	48 (14.1%)	31 (3.6%)	**< 0.001**
rPhl p 6, group 6 *Graminae*	42 (12.4%)	23 (2.7%)	**< 0.001**
nHev b 8, profilin	38 (11.2%)	20 (2.3%)	**< 0.001**
nOle e 7, LTP	31 (9.1%)	24 (2.8%)	**< 0.001**
rBet v 2, profilin	30 (8.8%)	17 (2%)	**< 0.001**
rPhl p11, ole e 1-related protein	22 (6.5%)	19 (2.2%)	**< 0.001**
rPhl p12, profilin	22 (6.5%)	15 (1.7%)	**< 0.001**
nArt v 1, defensin-like protein	16 (4.7%)	14 (1.6%)	**0.002**
rOle e 9, 1,3-beta-glucanase	12 (3.5%)	8 (0.9%)	**0.001**
rPla l 1, ole e 1-related protein	12 (3.5%)	13 (1.5%)	**0.026**
rChe a 1, ole e 1-related protein	8 (2.4%)	8 (0.9%)	0.088[1]
rPhl p 7, pocalcin	8 (2.4%)	3 (0.3%)	**0.003**[1]
nAmb a 1, pectate lyase	7 (2.1%)	11 (1.3%)	0.31
rBet v 4, pocalcin	6 (1.8%)	4 (0.5%)	**0.035**[1]
nSal k 1, pectin methylesterase	2 (0.6%)	4 (0.5%)	0.677
rPla a 1, invertase inhibitor	0	1 (0.1%)	1.000[1]

LTP, lipid transfer protein; - [1]Fisher's Exact test

Table III - *Frequency of positive test to different plant food allergenic molecules among Bet v 1 positive and Bet v 1 negative children.*

	Frequency (%) of positive test among		
Allergenic molecules	**Bet v 1 pos children**	**Bet v 1 neg children**	**P**
rMal d 1, PR-10 protein	276 (81.2%)	3 (0.3%)	**< 0.001**
rCor a1.04, PR-10 protein	246 (72.4%)	4 (0.5%)	**< 0.001**
rPru p 1, PR-10 protein	237 (69.7%)	2 (0.2%)	**< 0.001**
rGly m 4, PR-10 protein	127 (37.4%)	0 (0%)	**< 0.001**
rAra h 8, PR-10 protein	126 (37.1%)	0 (0%)	**< 0.001**
rPru p 3, lipid transfer protein (LTP)	90 (26.5%)	84 (9.7%)	**< 0.001**
rApi g 1, PR-10 protein	87 (25.6%)	2 (0.2%)	**< 0.001**
nJug r 3, lipid transfer protein (LTP)	84 (24.7%)	64 (7.4%)	**< 0.001**
nJug r 2, cupin	66 (19.4%)	26 (3%)	**< 0.001**
rAra h 9, lipid transfer protein (LTP)	58 (17.1%)	8 (0.9%)	**< 0.001**
nJug r 1, 2S albumin	54 (15.9%)	47 (5.4%)	**< 0.001**
nAct d 1, cysteine protease	52 (15.3%)	24 (2.8%)	**< 0.001**
rCor a 8, lipid transfer protein (LTP)	49 (14.4%)	39 (4.5%)	**< 0.001**
nAct d 8, PR-10 protein	42 (12.4%)	0 (0%)	**< 0.001**
nAra h 6, 2S albumin	26 (7.6%)	20 (2.3%)	**< 0.001**
nSes i 1, 2S albumin	25 (7.4%)	22 (2.5%)	**< 0.001**
rAra h 2, 2S albumin	23 (6.8%)	13 (1.5%)	**< 0.001**
nAct d 2, thaumatin-like protein	20 (5.9%)	27 (3.1%)	**0.026**
rAra h 1, cupin	17 (5%)	12 (1.4%)	**< 0.001**
nGly m 6, cupin	16 (4.7%)	18 (2.1%)	**0.013**
rTri a14, lipid transfer protein (LTP)	16 (4.7%)	10 (1.2%)	**< 0.001**
nGly m 5, cupin	15 (4.4%)	7 (0.8%)	**< 0.001**
rAna o 2, cupin	14 (4.1%)	19 (2.2%)	0.066
nAra h 3, cupin	9 (2.6%)	6 (0.7%)	**0.016**[1]
nTri aaA, alfa-amilase/trypsin inhibitor	5 (1.5%)	5 (0.6%)	0.156[1]
rBer e 1, 2S albumin	3 (0.9%)	3 (0.3%)	0.359[1]
nAct d 5, kiwellin	2 (0.6%)	1 (0.1%)	0.194[1]
nFag e 2, 2S albumin	0 (0%)	0 (0%)	-
rTri a19, omega-5 gliadin	0 (0%)	3 (0.3%)	0.563[1]

LTP, lipid transfer protein; - [1]Fisher's Exact test.

Table IV *- IgE levels to different pollen allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Bet v 1 pos children	**Bet v 1 neg children**	**p value**
rBet v 4, pocalcin	16.5 (2.4 - 34.4)	5.8 (3.5 - 9.5)	0.92
rPar j 2, LTP	14.8 (6.1 - 31.9)	5.7 (1.8 - 11.8)	**< 0.001**
nAmb a 1, pectate lyase	14.1 (1.5 - 40.1)	0.9 (0.6 - 3.4)	**0.018**
rPhl p5b, group 5 *Graminae*	9.0 (3.0 - 22.4)	6.4 (1.2 - 23.8)	0.38
rPhl p 1, group 1 *Graminae*	6.9 (2.7 - 18.4)	4.3 (1.1 - 21.2)	0.084
nOle e 1, olive group 1	6 (2.6 - 17.8)	1.9 (0.8 - 6.5)	**< 0.001**
rCor a1.01, **PR-10 protein**	5.7 (2.1 - 13.2)	0.5 (0.4 - 0.6)	**< 0.001**
rPhl p 7, pocalcin	5.3 (2.2 - 23.9)	4.2 (1.4 - 22.9)	0.92
nCup a 1, pectate lyase	4.8 (1.3 - 15.2)	1.9 (0.6 - 8.0)	**< 0.001**
nSal k 1, pectin methylesterase	4.2 (0.3 - 8.0)	7.8 (3.7 - 12.8)	0.64
rAln g 1, **PR-10 protein**	3.4 (1.1 - 8.4)	(-)	-
rBet v 2, profilin	3.1 (0.9 - 10.0)	8.9 (3.0 - 13.7)	0.09
rPhl p 2, group 2 *Graminae*	2.9 (1.5 - 8.3)	2.7 (0.8 - 7.9)	0.45
rMer a 1, profilin	2.9 (0.7 - 6.3)	8.1 (3.1 - 14.4)	**0.016**
rPla l 1, ole e 1-related protein	2.8 (0.8 - 10.8)	1.2 (0.7 - 8.7)	0.72
rPhl p11, ole e 1-related protein	2.7 (2.0 - 8.2)	2.7 (1.0 - 19.6)	0.70
nCyn d 1, group 1 *Graminae*	2.7 (0.9 - 9.2)	3.3 (1.2 - 13.9)	0.19
rPhl p12, profilin	1.7 (0.8 - 2.5)	3.3 (0.7 - 5.4)	0.54
nArt v 1, defensin	1.6 (0.6 - 4.4)	3.0 (1.0 - 5.2)	0.35
rPhl p 6, group 6 *Graminae*	1.5 (0.8 - 7.1)	3.3 (0.7 - 14.8)	0.34
nOle e 7, LTP	1.2 (0.7 - 7)	1.0 (0.5 - 2.3)	0.28
nPhl p 4, berberine bridge enzyme-like protein	1 (0.5 - 2.6)	1.3 (0.5 - 4)	0.31
nPla a 2, polygalacturonase	1 (0.5 - 1.6)	1 (0.5 - 1.3)	0.68
nArt v 3, LTP	1 (0.5 - 2)	0.8 (0.5 - 1.5)	0.74
nCry j 1, pectate lyase	1 (0.6 - 2)	0.8 (0.5 - 2.6)	0.82
rOle e 9, 1,3-beta-glucanase	0.9 (0.5 - 2.4)	1.3 (0.6 - 1.5)	0.97
rPla a 3, LTP	0.9 (0.6 - 2.4)	0.8 (0.6 - 1.4)	0.26
rChe a 1, ole e 1-related protein	0.8 (0.5 - 2.2)	0.8 (0.6 - 0.9)	0.96
rPla a 1, invertase inhibitor	(-)	1.2 (1.2 - 1.2)	-

LTP, lipid transfer protein;

Table V *- IgE levels to different plant food allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Bet v 1 pos children	**Bet v 1 neg children**	**p value**
nAct d 5, kiwellin	11.4 (10.6 - 12.3)	14.1 (14.1 - 14.1)	--
nAra h 3, cupin	6.3 (2.8 - 11.2)	1.8 (1.1 - 2.1)	**0.008**
rAra h 2, 2S albumin	5.1 (1.8 - 20.3)	5.1 (0.9 - 9.7)	0.25
nAra h 6, 2S albumin	4.5 (1 - 11.6)	2.5 (1.1 - 5.7)	0.36
rMal d 1, **PR-10 protein**	4.5 (1.6 - 11.0)	0.4 (0.3 - 0.6)	**0.006**
rCor a1.04, **PR-10 protein**	4.3 (1.7 - 8.8)	1.5 (0.5 - 2.8)	0.055
nAct d 2, thaumatin-like protein	3.1 (1.3 - 5.9)	2.1 (0.7 - 2.9)	**0.021**
rAra h 1, cupin	3.1 (1.5 - 7.4)	2.3 (1.5 - 5.2)	0.55
rPru p 1, **PR-10 protein**	3.0 (1.1 - 8.0)	0.7 (0.4 - 1.0)	0.08
nGly m 6, cupin	2.6 (0.8 - 6.8)	0.8 (0.7 - 2.5)	0.17
nJug r 1, 2S albumin	2.5 (1.4 - 7.9)	1.6 (1.1 - 5.6)	0.14
rGly m 4, **PR-10 protein**	2.0 (0.8 - 5.5)	(-)	-
nSes i 1, 2S albumin	1.9 (0.7 - 3.9)	1.3 (0.7 - 6.8)	0.94
rAra h 8, **PR-10 protein**	1.7 (0.8 - 3.5)	(-)	-
nJug r 3, LTP	1.4 (0.8 - 3.5)	0.9 (0.5 - 1.9)	**0.032**
rApi g 1, **PR-10 protein**	1.4 (0.8 - 3.0)	1.4 (0.7 - 2.1)	0.74
nAct d 1, cysteine protease	1.3 (0.8 - 2.4)	1.0 (0.6 - 3.3)	0.46
nGly m 5, cupin	1.3 (0.8 - 4.1)	1.3 (0.4 - 3.7)	0.78
rAra h 9, LTP	1.15 (0.6 - 3.1)	0.8 (0.6 - 1.4)	0.07
nTri aaA, alfa-amilase/trypsin inhibitor	1.1 (0.4 - 1.3)	1.6 (0.7 - 2.2)	0.21
rPru p 3, LTP	1.1 (0.6 - 2.8)	1.0 (0.6 - 2.1)	0.56
rAna o 2, cupin	1.1 (0.4 - 2.8)	1 (0.4 - 2.1)	0.69
rCor a 8, LTP	1.0 (0.5 - 1.9)	0.7 (0.4 - 1.3)	0.10
nJug r 2, cupin	0.9 (0.5 - 1.4)	1.2 (0.7 - 2.4)	0.09
rBer e 1, 2S albumin	0.9 (0.3 - 3.5)	1.6 (0.6 - 2)	-
rTri a14, LTP	0.8 (0.5 - 1.8)	1.1 (0.9 - 1.7)	0.30
nAct d 8, **PR-10 protein**	0.7 (0.5 - 1.2)	(-)	-
nFag e 2, 2S albumin	11.4 (10.6 - 12.3)	(-)	-
rTri a19, omega-5 gliadin	6.3 (2.8 - 11.2)	1 (0.3 - 1.6)	-

LTP, lipid transfer protein;

Multiple correspondence analysis was performed to evaluate the possible mutual interrelationships among different PR-10s on the basis of IgE reactivity. Since this technique places variables based on their levels of reciprocal relationship (i.e., highly related variables are close to each other, while unrelated variables are placed far away from each other), we found that Bet v 1 positivity is frequently associated to sensitization to all the other pollen-derived molecules (i.e., Cor a 1.01, Aln g 1) and to some plant-food allergens (i.e. Mal d 1, Pru p 1 and Cor a 1.04). A different behavior was found for the remaining pollen-derived

molecules: Gly m 4 positivity was in fact associated to Ara h 8 and Api g 1positivity, whereas Act d 8 is less associated to other PR-10s (**figure 1**).

In addition, we analysed the frequency of sensitization and the IgE level to other molecules belonging the most common allergens in our region, i.e. house dust mites, cat, dog, caw milk, and egg, as reported in **table VI** and **VII**, respectively. Interestingly, Bet v 1 positive children showed higher frequency and serum IgE level than Bet v 1 negative children about most of the molecules.

Figure 1 - *Multiple correspondence analysis plot showing the mutual interrelationships between PR-10s (each molecule is represented by one dot) in terms of IgE reactivity (positivity/negativity). The horizontal axis (F1) explains 97.66% of total variability (inertia); the vertical axis (F2) explains 0.04% of total variability. See methods' section for details.*

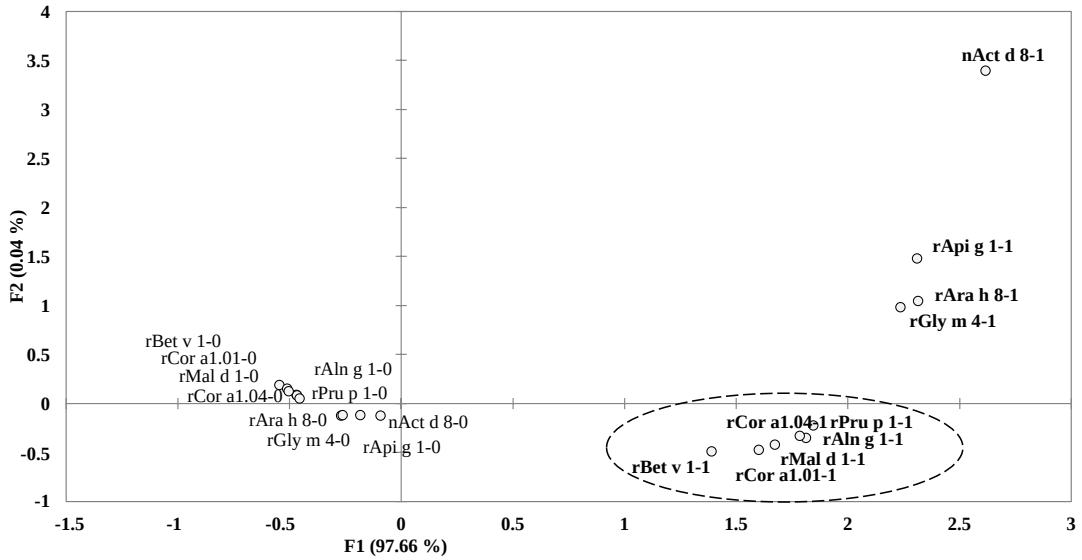

Table VI - *Frequency of positive test to different other allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Frequency [N (%)] of positive test among		
Allergenic molecules	**Bet v 1 pos children**	**Bet v 1 neg children**	**P**
rDer f 2, mite group 2 family	210 (61.8%)	291 (33.6%)	**< 0.001**
rFel d 1, uteroglobin	200 (58.8%)	158 (18.3%)	**< 0.001**
nDer p 2, mite group 2 family	197 (57.9%)	276 (31.9%)	**< 0.001**
nDer f 1, cystein protease	178 (52.4%)	255 (29.5%)	**< 0.001**
nDer p 1, cystein protease	178 (52.4%)	241 (27.9%)	**< 0.001**
rCan f 1, lipocalin	82 (24.1%)	70 (8.1%)	**< 0.001**
rCan f 5, arginin esterase	59 (17.4%)	45 (5.2%)	**< 0.001**
rLep d 2, mite group 2 family	51 (15.0%)	64 (7.4%)	**< 0.001**
nGal d 2, ovalbumin	34 (10.0%)	50 (5.8%)	**0.010**
rDer p 10, tropomyosin	27 (7.9%)	32 (3.7%)	**0.002**
nGal d 1, ovomucoid	25 (7.4%)	37 (4.3%)	**0.030**
nGal d 3, conalbumin	23 (6.8%)	30 (3.5%)	**0.012**

	Frequency [N (%)] of positive test among		
Allergenic molecules	**Bet v 1 pos children**	**Bet v 1 neg children**	**P**
rCan f 2, lipocalin	22 (6.5%)	18 (2.1%)	**< 0.001**
nBos d 4, alfa-lactalbumin	19 (5.6%)	47 (5.4%)	0.92
rFel d 4, lipocalin	16 (4.7%)	17 (2.0%)	**0.009**
nFel d 2, serum albumin	15 (4.4%)	16 (1.8%)	**0.011**
nBos d 8, casein	14 (4.1%)	40 (4.6%)	0.70
nCan f 3, serum albumin	14 (4.1%)	12 (1.4%)	**0.003**
nBos d 5, beta-lactoglobulin	13 (3.8%)	41 (4.7%)	0.49
nBos d 6, serum albumin	8 (2.4%)	24 (2.8%)	0.68
nBos d I, trasferrin	5 (1.5%)	9 (1.0%)	0.554[1]
nGal d 5, serum albumin	5 (1.5%)	10 (1.2%)	0.773[1]

[1]Fisher's Exact test

Table VII *- IgE levels to different other allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Bet v 1 pos children	**Bet v 1 neg children**	**p value**
rDer f 2, mite group 2 family	27.6 (11.2 - 55.4)	17.3 (5.5 - 43.1)	**< 0.001**
nDer f 1, cystein protease	12.5 (5.0 - 26.4)	7.3 (3.0 - 19.2)	**< 0.001**
nDer p 2, mite group 2 family	11.3 (4.4 - 24.2)	7.8 (2.9 - 17.7)	**0.002**
nDer p 1, cystein protease	7.8 (3.2 - 18.1)	5.5 (2.2 - 11.8)	**0.010**
rFel d 1, uteroglobin	7.1 (2.4 - 17.7)	4.45 (1.4 - 13.5)	**0.010**
rFel d 4, lipocalin	4.6 (2.4 - 9.6)	1.9 (0.6 - 8.5)	0.28
rCan f 2, lipocalin	3.1 (1.3 - 13.4)	4.6 (1.1 - 10.7)	0.90
rCan f 1, lipocalin	3.0 (1.1 - 12.5)	3.0 (1.1 - 10.6)	0.84
rDer p 10, tropomyosin	2.7 (0.8 - 18.9)	2.8 (0.6 - 14.2)	0.91
nBos d 4, alfa-lactalbumin	2.7 (0.95 - 7.45)	1.6 (0.7 - 4.05)	0.29
nBos d 5, beta-lactoglobulin	2.7 (0.75 - 15.1)	1.2 (0.5 - 3.3)	0.13
nBos d 8, casein	2.65 (0.5 - 12.25)	1.1 (0.65 - 3.9)	0.46
rCan f 5, arginin esterasi	2.2 (0.8 - 5.8)	1.8 (0.9 - 5.3)	0.97
nGal d 3, conalbumin	1.7 (0.8 - 7)	1.9 (0.7 - 6.6)	0.80
rLep d 2, mite Group 2 family	1.4 (0.8 - 4.5)	1.7 (0.8 - 7.2)	0.40
nBos d 6, serum albumin	1.2 (0.6 - 2.9)	1.1 (0.6 - 2.2)	0.78
nGal d 2, ovalbumin	1.0 (0.6 - 2.85)	0.8 (0.45 - 3.8)	0.60
nGal d 1, ovomucoid	1.0 (0.5 - 2.2)	1.8 (0.7 - 6.8)	**0.026**
nFel d 2, serum albumin	0.8 (0.6 - 5.2)	0.6 (0.4 - 1.5)	0.27
nCan f 3, serum albumin	0.8 (0.4 - 5.1)	1.25 (0.4 - 2.5)	0.76
nGal d 5, serum albumin	0.6 (-)	2.3 (0.8 - 22.6)	0.16
nBos d, lactoferrin-trasferrin	0.4 (-)	2.1 (1.5 - 6.3)	**0.007**

different behavior was found for the remaining pollen-derived molecules: Gly m 4 positivity was in fact associated to Ara h 8 and Api g 1positivity, whereas Act d 8 is less associated to other PR-10s (**figure 1**).
In addition, we analysed the frequency of sensitization and the IgE level to other molecules belonging the most common allergens in our region, i.e. house dust mites, cat, dog, caw milk, and egg, as reported in **table VI** and **VII**, respectively. Interestingly, Bet v 1 positive children showed higher frequency and serum IgE level than Bet v 1 negative children about most of the molecules.

Discussion

The assessment of IgE to pan-allergens is useful in the allergy work-up. In this context, a clinical question is: can pan-allergens affect the sensitization pattern? A previous study, conducted in adults, showed that sensitization to a pan-allergen (i.e. Bet v 1, Pru p 3, and Bet v 2) entails higher odds to have other sensitizations (14). In addition, the co-sensitization pattern depended on the basis of the sensitizing pan-allergen family primer. As birch allergy is very common in Genoa, curiously a birch-free geographical area (16), we focused our attention on Bet v 1, to test the hypothesis that sensitization to the major allergen of PR-10 family, such as Bet v 1, could affect the sensitization pattern in children.
The current study shows that Bet v 1 sensitization is significantly associated with sensitization to other PR-10 allergens, both pollens and fruits/vegetables. In contrast, children not sensitized to Bet v 1 very rarely are sensitized to other PR-10 allergens, including pollens and fruits/vegetables. Interestingly, both age and gender did not significantly impact on findings. The most common profilin was rMer a 1 in our sample. Curiously, Bet v 4 induced the highest serum level, but this findings could be due to the very low number of subjects, thus out-layers could interfere with results. This finding may be obviously explained by the homology shared by PR-10 molecules. More interestingly, Bet v 1 sensitization is also associated to sensitization to allergens belonging to other families, namely LTP-family, 2S albumin-family, cupin-family, polcalcin-family, and also to other pollens, mainly grasses and olive tree. This phenomenon could be considered as a "priming" effect where the primary Bet v 1 sensitization may promote the development of successive sensitizations to other allergens. Probably this effect could be initially limited to PR-10 family and then extended also to other allergen clusters. This finding is underlined by the multivariate analysis, that clearly highlights the close homologous behavior of PR-10 sensitization: the preserved molecular sequence of pollen and food molecules may explain the high frequency of co-sensitization patterns. Interestingly, the effect of Bet v 1 sensitization has an impact also on other molecules belonging to other allergens, including house dust mites, cat, dog, caw milk, and egg. This finding could mean that the pan-allergen Bet v 1 could be a sensitization primer, even though further longitudinal studies should confirm this cross-sectional outcome.
So, the current pediatric study provided findings that are consistent with a previous one conducted on adult patients living in the same geographic area. Moreover, a recent study conducted on allergic patients living in central and southern Italy (birch-free area), demonstrated that there are specific relationships between sensitization patterns and clinical characteristics in subjects with Bet v 1 sensitization (17). In this regard, previous studies investigated the relevance of *Betulaceae* pollens counts about sensitization pattern and clinical expression (18,19). In fact, allergy to *Betulaceae* represents a primary cause of sensitization and allergic symptom severity in our geographic area.
Anyway, the current study had some limitations: it was retrospectively conducted on a selected patient population sample, subjects referring for serologic assessment, there was no follow-up, and there are no clinical data. This issue is particularly relevant, as sensitization does not always correspond to allergy: this fact probably further reduces the percentages of subjects really "positive" to tests, such as truly allergic. In addition, this study did not consider possible confounding factors, such as passive smoking status, parasite infestation, environmental exposures, and seasonal variations. Finally, it has to be considered that outcomes from ISAC may be not completely precise as many factors may interfere, such as the amount of allergen in the assay, the semi-quantitative analysis, and the chemical-physical characteristics of allergen molecules. Therefore, there is need to conduct cohort studies and long-term follow up trials to confirm these preliminary findings. However, the strength of the present study may be represented by the large size of the sample.
Another relevant issue is the possibility of investigating the potential role exerted by other pan-allergenic molecules, mainly concerning Pru p 3, the major allergen belonging to the lipid transfer protein family. In this regard, a study is ongoing in our geographic area, analyzing data deriving from the more precise ImmunoCap method.
Moreover, this study was cross-sectional, reflecting a single time point without analysing the evolution of sensitization over time. However, we very recently published two studies, concerning the present cohort and adults, analyzing the ISAC findings for pollen and food sensitizations (20,21). We demonstrated that Bet v 1 sensitization frequency progressively increased from 2% at < 2 years of age to a peak of 43.3% at 20 years of age. Equally, IgE serum levels progressively increased from 0.5 ISU at < 2 years of age to a peak of 8 ISU at 20 years. Furthermore, Cor a 1 sensitization frequency increased from < 1% at < 2 years of age to a peak of about 40% at 20 years. Likewise, IgE serum levels progressively increased from 4 ISU to a peak of 13 ISU at about 10 years.

It has also to be noted that our findings are consistent with previous studies that highlighted Bet v 1 as the strongest or most prevalent allergen in the PR-10 group (22,23). On the other hand, we focused our attention on Bet v1 as potential primer, but also Der f 2 and Fel d 1 could be protagonists in priming sensitization. In this regard, there is a longitudinal study that is ongoing. Another unmet need concerns the curious preeminence of Bet v 1 sensitization on other PR-10 molecules, including Cor a 1; molecular studies should be conducted to provide adequate explanation.

In conclusion, the present study demonstrates that Bet v 1 sensitization may significantly affect the sensitization pattern in children living in Genoa, a Mediterranean city located in a birch-free area.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Fasce L, Tosca MA, Baroffio M, et al. Atopic infants with wheezing start always with monosensitization. All Asthma Proc 2007; 28:449-453.
2. Melioli G, Marcomini L, Agazzi A, et al. The IgE repertoire in children and adolescents resolved at component level: A cross-sectional study. Pediatr Allergy Immunol 2012; 23:433-440.
3. Arbes SJ, Jr., Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 2005; 116:377-383.
4. Baatenburg de Jong A, Dikkeschel LD, Brand PL. Sensitization patterns to food and inhalant allergens in childhood: a comparison of non-sensitized, monosensitized, and polysensitized children. Pediatr Allergy Immunol 2011; 22:166-171.
5. Sastre J. Molecular diagnosis in allergy. ClinExp Allergy 2010; 40:1442-1460.
6. Sastre J, Landivar ME, Ruiz-Garcia M, et al. How molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area. Allergy 2012; 67:709-711.
7. McKenna OE, Asam C, Araujo GR, et al. How relevant is panallergen sensitization in the development of allergies? Pediatr Allergy Immunol. 2016; 27:560-568.
8. Di Rienzo Businco A, Sfika I, Bianchi A, et al. Prevalence and Clinical Relevance of IgE Sensitization to Profilin in Childhood: A Multicenter Study. Int Arch Allergy Immunol 2015; 168:25-31.
9. Mastrorilli C, Tripodi S, Caffarelli C, et al. Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification. Allergy 2016; 71:1181-1191.
10. Jensen-Jarolim E. Happy 25th birthday, Bet v 1! WAO Journal 2014; 7:14-17.
11. Breiteneder H, Pettenburger K, Bito A, et al. The gene coding for the major birch pollen allergen Bet v I, is highly homologous to a pea disease resistance response gene. EMBO J 1989; 8:1935-1938.
12. Ciprandi G, Comite P, Ferrero F, Bignardi D, Minale P, Voltolini S, Troise C, Mussap M. Birch allergy and oral allergy syndrome: the practical relevance of serum IgE to Bet v 1. Allergy Asthma Proc 2016; 37:43-9.
13. Ciprandi G, Comite P, Ferrero F, et al. Can serum Bet v 1IgE measurement distinguish between sensitization and allergy? Int Forum Allergy Rhinol 2015; 5:1151-1155.
14. Ciprandi G, Comite P, Bruzzone M, Fontana M. Can pan-allergens affect the sensitization pattern? Immunobiology 2017; 222:726-9.
15. Greenacre M, Blasius J. Multiple Correspondence analysis and related methods. Chapman and Hall/CRC London 2006: 1-581
16. Ciprandi G, Comite P, Mussap M, et al. Profiles of birch sensitization (Bet v 1, Bet v 2, Bet v 4) and oral allergy syndrome across Italy. J Inv All Clin Immun 2016; 26:244-8.
17. Scala E, Abeni D, Cecchi L, et al. Molecular recognition profiles and clinical patterns of PR-10 sensitization in a birch-free Mediterranean area. Int Arch Allergy immunol 2017; 173:138-146.
18. Negrini AC, Negrini S, Giunta V, Quaglini S, Ciprandi G. Thirty year survey on airborne pollen concentrations in Genoa (Italy): relationship with sensitizations, meteorological data, and air pollution. Am J Rhinol Allergy 2011; 25:232-241.
19. Ruffoni S, Passalacqua G, Ricciardolo F, Furgani A, Negrini AC, DeAmici M, Ciprandi G. A 10-year survey on asthma exacerbations: relationships among emergency medicine calls, pollens, weather, and air pollution. Rev Franc Allergologie 2013; 44:111-116.
20. Tosca MA, Silvestri M, Olcese R, Pistorio A, Rossi GA, Ciprandi G. The impact of age on serum allergen-specific IgE to inhaled molecular components. Allergologia et Immunopathologia 2017; 45265-45271.
21. Tosca MA, Silvestri M, Olcese R, Sacco O, Pistorio A, Rossi GA, Ciprandi G. Allergen-specific IgE to food molecular components and age: from early childhood to adulthood. Allergol Immunopathol 2017; 45:87-89.
22. Tolkki L, Alanko K, Petman L, Skydtsgaard MB, Milvang PG, Seppälä U, Ranki A. Clinical characterization and IgE profiling of birch (Betula verrucosa)--allergic individuals suffering from allergic reactions to raw fruits and vegetables. J Allergy Clin Immunol Pract 2013; 1:623-631.
23. Siroux V, Lupinek C, Resch Y, Curin M, Just J, Keil T, Kiss R, et al. Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: The EGEA study. J Allergy Clin Immunol 2017; 139:643-654.

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Infectious etiology of chronic diarrhea in patients with primary immunodeficiency diseases

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Key words

primary immunodeficiency disease; common variable immunodeficiency; hyper-IgM syndrome; severe combined immunodeficiency; X-linked agammaglobulinemia; infection

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Summary

Background. *Primary immunodeficiency diseases (PIDs) are life-threatening disorders, which manifest commonly with gastrointestinal (GI) signs, mainly as chronic diarrhea.* ***Objective.*** *To investigate and compare infectious etiology of chronic diarrhea in different PIDs.* ***Patients and methods.*** *Assessing clinical features, obtaining immunological profiles, as well as characterizing infectious etiology of diarrhea were performed in 38 PID patients with chronic diarrhea. Stool samples and/or biopsy specimens were checked using culture, microscopic examination, RT-PCR, and PCR, as appropriate. The patients were diagnosed to have common variable immunodeficiency (CVID), severe combined immunodeficiency (SCID), X-linked agammaglobulinemia (XLA), and hyper-IgM (HIgM) syndrome.* ***Results.*** *In 32 patients we identified 41 infectious agents including 16 parasitic (39.0%, the most common Giardia lamblia), 11 bacterial (26.8%, the most common salmonella spp), 8 viral (19.5%, the most frequent group A rotavirus), and 6 fungal organisms (14.7%, the most common Candida albicans). From 6 of the patients, no infectious agent was isolated. In CVID bacteria and parasites, in SCID bacteria and viruses, in XLA parasites, and in individuals with HIgM syndrome parasites were the leading causes of chronic diarrhea. Infection with giardia and cryptosporidium were more frequent in XLA and HIgM, respectively.* ***Conclusion.*** *The current study suggests considering both usual and unusual pathogens in laboratory investigation and in the empiric treatment of chronic diarrhea. Opportunistic pathogens should be taken into account when no other pathogen is identified, especially in patients on long-term treatment or prophylaxis with antifungals/antibiotics and in those from geographical locations that favor pathogenicity of these organisms.*

Introduction

Primary immunodeficiency diseases (PIDs) are a heterogeneous group of genetic disorders affecting the development and/or function of the immune system with an overall prevalence of 1 in 10,000 live births (1). The patients are susceptible to autoimmune diseases, malignancies, as well as infections, especially of respiratory and gastrointestinal (GI) tracts. GI problems are the second most common manifestations of PID after respiratory problems and may be the first presentations in some cases. Among different GI complications, chronic diarrhea is the most common, arising from non-infectious as well as infectious etiologies. PID patients are more prone to diarrhea, due to a wide range of pathogens, and less responsive to standard therapies than healthy individuals. Moreover, intestinal damages and loss of nutrients and proteins worsen immune status and trigger a vicious circle that deteriorates immune defects. Elucidating infectious agents responsible for diarrhea in these patients, helps both precise laboratory investigation of suspected pathogens and subsequent correct diagnosis and treatment, leading to more survival, less mortality, and better life quality with fewer organ damages (2-4). The aim of the present study was to determine infectious etiology of chronic diarrhea in PID individuals.

Patients and methods

Patients

Iranian Primary Immunodeficiency Registry (IPIDR) has been active since 1997, and 1640 cases with a variety of PIDs were registered up to the end of 2014 (5). Within patients referred to Children's Medical Center (CMC) hospital in Tehran from January 2013 through June 2014, PID was diagnosed in 147 individuals. Among them, 38 patients with chronic diarrhea were included in the study. The study was approved by the ethics committee of Tehran University of Medical Sciences in accordance with the ethical standards. The participating patients or their parents were also given verbal information before taking their written informed consent. The diagnosis of PID was based on the European Society for Immunodeficiencies and the Pan-American Group for Immunodeficiency (ESID/PAGID) criteria (6). Chronic diarrhea was defined as the production of loose stool more than 10 mL/kg/day in infants or more than 200 g in other ages that last more than 2 weeks (7).

Data Collection

A questionnaire was designed to obtain information, including patient's demographic information such as age, sex, date of birth, place of birth, diagnosis of PID, course of PID, immunological laboratory results, and diarrhea data. All of the questionnaires were completed by physicians involved in the care of the reported patients.

Laboratory testing

Immunological laboratory tests were performed. Moreover, stool samples and biopsy specimens were taken and checked for bacterial culture and also looked for *Clostridium difficile* (*C. difficile*) toxin, *Clostridium perfringens* (*C. perfringens*) toxin, parasites, and fungi/yeast-like organisms. Enteroviruses as well as group A rotavirus was checked using RT-PCR method, and cytomegalovirus (CMV) using PCR.

Statistical analysis

Fisher's exact test and chi-square tests were used for 2 × 2 comparison of categorical variables, whereas t-tests and one-way ANOVA were used to compare numerical variables. Statistical analysis was performed using the SPSS software package, version 17 (SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered significant.

Results

Demographic and clinical information

From 147 PID patients evaluated, 38 patients fulfilled the criteria for chronic diarrhea (81.6% male) and enrolled in the study during the 18 month period of the study. No sign of dysentery was observed. The median age of patients at the time of the study was 12 (3.3-22.0) years. According to ESID/PAGID criteria (6), patients were diagnosed as having common variable immunodeficiency (CVID) (n = 12; 31.6%), severe combined immunodeficiency (SCID) (n = 11; 28.95%), *X-linked agammaglobulinemia* (XLA) (n = 11; 28.95%), and hyper-IgM (HIgM) syndrome (n = 4; 10.5%). Statistical analysis did not show any significant correlation between age at first diarrhea and age at onset or age at diagnosis of PID. Demographic information, as well as immunological laboratory data and diarrhea information of the study population, are illustrated in **tables I** and **II**, respectively.

Table I *- Demographic data of PID patients with chronic diarrhea.*

Parameter	Results
sex, m/f	31/7
age, y	12.0 (3.3 - 22.0)
age at onset of PID symptoms, y	0.6 (0.2 - 1.8)
age at diagnosis of PID, y	3.0 (1.0 - 7.5)
age at first diarrhoea, y	2.00 (0.5 - 7.3)

PID, primary immunodeficiency; f, male; f, female; y, year. For quantitative parameters, the median is shown (with 25th and 75th percentiles).

Table II *- Clinical and immunological data of PID patients with chronic diarrhea.*

Diagnosis (number of patients)	Ig level (mg/dL); median (IQR)			CD (%); median (IQR)				Age at first diarrhea, y; median (IQR)
	IgM	IgG	IgA	$CD3^+$	$CD4^+$	$CD8^+$	$CD19^+$	
CVID (12)	18.0 (4.0 - 80.0)	114.0 (68.0 - 570.0)	5.0 (0 - 13.0)	74.0 (65.5 - 82.0)	23.0 (10.5 - 29.0)	52.0 (36.5 - 62.5)	6.5 (2.7 - 13.3)	3.0 (0.5 - 13.0)
SCID (11)	27.0 (10.0 - 150.0)	156.0 (99.0 - 570.0)	7.0 (0.0 - 129.3)	2.9 (0.5 - 10.9)	1.4 (0.1 - 19.5)	1.8 (0.4 - 11.8)	32.7 (0.2 - 86.3)	0.5 (0.3 - 0.8)
XLA (11)	20.0 (0.0 - 26.0)	112.0 (10.0 - 290.0)	11.5 (0.5 - 39.5)	90.4 (71.9 - 93.2)	44.9 (25.3 - 51.3)	37.5 (25.5 - 45.9)	0.2 (0.1 - 1.2)	2.0 (1.0 - 9.3)
HIgM (4)	256.0 (94.0 - 727.5)	56.0 (3.8 - 205.8)	10.0 (3.0 - 22.0)	76.5 (65.0 - 83.5)	42.0 (30.0 - 56.3)	29.5 (21.0 - 40.3)	15.1 (8.9 - 18.2)	2.5 (0.5 - 4.5)
p-value	0.059	0.325	0.754	< 0.001[1]	0.002[1]	< 0.001[1]	0.002[1]	0.082

CVID, common variable immunodeficiency; HIgM, hyper IgM syndrome; PID, primary immunodeficiency; SCID, severe combined immunodeficiency; XLA, X-linked agammaglobulinemia. For quantitative parameters the median is shown (with 25th and 75th percentiles). Kruskal-Wallis test was used to compare parameters.

[1]A p-value of 0.05 or less is statistically significant.

Infectious etiology

From 32 PID patients, 41 infectious agents were isolated and recognised as causes of diarrhea, including 16 parasitic (39.0%, the most common *Giardia lamblia; G. lamblia*), 11 bacterial (26.8%, the most common *Salmonella* spp.), 8 viral (19.5%, the most common group A rotavirus), and 6 fungal/yeast like agents (14.7%, the most frequent *Candida albicans; C. albicans*) (**table III**). Detailed information on infectious agents in each PID is represented in **table III**. From 6 of the patients (3 SCID and 3 CVID individuals) no infectious agent was isolated, however, in CVID patients, two were diagnosed with IBD and another with celiac disease.

Regarding infectious etiology of diarrhea within each PID group, 16 infectious agents were isolated from CVID patients. Bacterial and parasitic organisms were leading pathogens, and viral and fungal agents were in the second order of frequency. From SCID subjects, 8 pathogens were isolated; among them, bacteria and viruses were the most common. However, a fungus/yeast was also isolated. In XLA individuals 12 infectious agents were recognized; among them, parasites were obviously dominant, followed by fungal, bacterial, and viral pathogens. And finally, from HIgM patients 5 organisms were isolated as causes of diarrhea, while parasites were dominant followed by viral etiology (**table III**). Fisher exact test showed that *Giardia* infections were significantly more frequent in XLA compared with other PIDs (p = 0.001). Moreover, the frequency of *Cryptosporidium* infection was significantly higher in HIgM compared with other PIDs (p = 0.009).

Discussion

GI problems, especially chronic infectious diarrhea, are among common manifestations in PID patients necessitating unraveling underlying etiology. Different parasitic, bacterial, viral, and fungal/yeast-like organisms have been reported as responsible causes. Defects in nutrient absorption caused by diarrhea in PID patients, via impairing structure and function of immune components, poses affected individuals to the higher risk of immune disturbances. Considering the consequences of GI complications for PID patients, and the necessity for correct diagnosis with subsequent proper treatment, we aimed to investigate infectious etiology of chronic diarrhea in different PIDs (3,8).

In our CVID patients, frequent pathogens were bacterial (including *Salmonella* spp.) and parasitic (including *G. lamblia*), and with less frequencies, viral (group A rotavirus and CMV) and fungal (*C. albicans*) (**table III**). Cunningham-Rundles and Bodian reported parasitic (*Giardia*) and bacterial pathogens (*Campylobacter* and *Salmonella* spp.) as the main causes of diarrhea in CVID patients, however, a viral organism (CMV) was also isolated (9). McCabe reported *Giardia* as infectious agent associated with CVID (10). In our previous cohort of 83 Iranian humoral immunodeficient patients, including CVID cases, *G. lamblia* followed by *Salmonella* spp. were prevalent pathogens (11). In a French study on CVID patients, bacterial (including *Salmonella* spp.) followed by parasitic pathogens (*Giardia*) were leading causes (12). Our results are in accordance with organisms reported by other studies. Moreover, less frequent pathogens were isolated from our patients such as enterotoxigenic *C.*

Table III *- Infectious etiology of chronic diarrhea in different PID patients.*

		Number of infectious agents in each type of PID				
Infectious organism	total	CVID	SCID	XLA	HIgM	p-value
parasites	16	5	-	7	4	< 0.001[1]
Giardia lamblia	9	1	-	7	1	0.001[1]
Cryptosporidium spp.	2	-	-	-	2	0.009[1]
Blastocystis hominis	1	-	-	-	1	0.1
Fasciola spp.	1	1	-	-	-	1.0
hookworm	1	1	-	-	-	1.0
Schistosoma spp.	1	1	-	-	-	1.0
Trichostrongylus spp.	1	1	-	-	-	1.0
bacteria	11	6	4	1	-	0.09
Salmonella spp.	5	2	2	1	-	1.0
Clostridium difficile	1	1	-	-	-	1.0
Pseudomonas aeruginosa	2	-	2	-	-	0.35
Enterotoxigenic Clostridium perfringens	1	1	-	-	-	1.0
Fusobacterium spp.	1	1	-	-	-	1.0
Shigella spp.	1	1	-	-	-	1.0
viruses	8	3	3	1	1	0.72
group A rotavirus	5	1	3	-	1	0.18
enterovirus (non-polio)	1	1	-	-	-	1.0
poliovirus (VDPV)	1	-	-	1	-	0.64
cytomegalovirus	1	1	-	-	-	1.0
fungi/yeasts	6	2	1	3	-	0.66
Candida albicans	5	2	1	2	-	1.0
Trichosporon spp.	1	-	-	1	-	0.68

CVID, common variable immunodeficiency; HIgM, hyper IgM syndrome; PID, primary immunodeficiency; SCID, severe combined immunodeficiency; VDPV, vaccine-derived poliovirus; XLA, X-linked agammaglobulinemia.

[1]A p-value of 0.05 or less is statistically significant.

perfringens and *Fusobacterium* spp. among bacteria, and *C. albicans* as fungus. However, our patients were children or young adults (not older than 22 years), and this could be a bias at least for CVID patients.

In SCID individuals we found bacterial (including *Salmonella* spp.) and viral (group A rotavirus) pathogens as the main etiology (**table III**). Other studies reported various viral etiologies including rotaviruses, CMV, astroviruses and noroviruses from SCID individuals (8,13-15). Bacterial etiology is another reported cause of diarrhea in SCID patients (3). We also observed, as opportunistic causes of diarrhea, *Pseudomonas aeruginosa (P. aeruginosa)* among bacteria, and *C. albicans* among fungi.

In our XLA patients, parasites (the only found organism was *G. lamblia*) obviously predominate, whereas with a less frequency fungal, bacterial (*Salmonella*), and viral pathogens (vaccine-derived poliovirus; VDPV) were also isolated (**table III**). Two relatively uncommon pathogens isolated from our patients were *C. albicans* and *Trichosporon* spp. belonging to the fungal/yeast-like type of organisms. *Giardia* infection was significantly more frequent in XLA compared with other PIDs. In our previous study, we observed *Giardia* as the sole infectious agent in XLA patients (11). In another cohort of Iranian XLA patients, we isolated bacteria (including *Shigella* spp.), followed by parasites (including *G. lamblia*) as causative organisms (16). In an American cohort of XLA individuals, parasites (*G. lamblia*), followed by bacteria (including *Salmonella* spp.), and viruses (rotavirus, enterovirus) were isolated (17).

In the current study, in HIgM individuals parasitic pathogens were including *G. lamblia, Blastocystis hominis* and *Cryptosporidium* spp. However, a viral pathogen (group A rotavirus) was also isolated. *Cryptosporidium* infection had a significantly higher frequency in HIgM compared with other PIDs. Our results are in accordance with the results reported by Winkelstein et al., who observed parasites (*Cryptosporidium* and *G. lamblia*) as the main infectious etiology of diarrhea, followed by viruses (rotavirus) and bacteria in HIgM individuals (18).

Altogether, our results regarding infectious etiology of chronic diarrhea are in line with above-mentioned studies. In CVID patient's parasites and bacteria, in SCID viruses and bacteria, in XLA individuals parasites and fungi, and in patients with HIgM syndrome parasites, were most frequent etiologies. However, some differences appear: 1) order of organism types responsible for diarrhea in some PIDs. For example, in our SCID patients bacterial and viral pathogens had equal contribution in eliciting diarrhea, but in some other studies viral pathogens predominate. This could be due to some limitations in our study for laboratory investigation of all possible viral causes of diarrhea. 2) We isolated the same types of pathogens reported by previous studies, but within these pathogens some opportunistic or unusual organisms were also identified, including enterotoxigenic *C. perfringens, P. aeruginosa*, and *Fusobacterium* spp. among bacteria. 3) Moreover, we identified *C. albicans* and *Trichosporon* spp., two relatively unusual causes of diarrhea as fungal/yeast etiology.

Though not frequently, the unusual infectious agents were reported by other studies, and well documented as opportunistic causes of diarrhea even in immunocompetent individuals. Enterotoxigenic *C. perfringens* has been identified as the cause of both sporadic and antibiotic-associated diarrhea (AAD) (19-23) and its role in eliciting experimental diarrhea was also confirmed (24). *P. aeruginosa* has been isolated from the feces of diarrheal patients with the defective immune system or who were hospitalized or received antibiotics or who had an underlying disease (3,25,26). *Candida* spp., especially *C. albicans*, have been isolated as the pathogen in sporadic (27-33) as well as AAD (34), both in immunocompetent individuals and in patients with immunodeficiency (3,35-36). Antifungal treatment leads to resolution of diarrhea from Candida (37,38). The role of *Trichosporon* in eliciting diarrhea has been documented in sporadic cases (27,33), in hosts with defective immune function (35), in the immunosuppressed host (39), and in bone marrow transplanted patient who had received antifungal prophylaxis and several antibiotics (40).

In fact, opportunistic pathogens are inhabitants of human natural environment, and live in healthy individuals as part of normal flora of some parts of the body, including intestine. Conditions such as immunodeficiency, frequent or prolonged hospitalization, prophylaxis or frequent/prolonged treatment with antibiotic or antifungal agents, and the presence of an underlying disease favour pathogenicity of these organisms. Within these risk factors immunodeficiency, which was the case in our patients, per se favors pathogenicity of opportunistic agents, while other mentioned risk factors often coexist with immunodeficiency in immunocompromised hosts. Indeed, PID patients experience repeated or prolonged hospitalization which expose them to various pathogens, including opportunistic ones carried by other patients, staff, medical devices, and the environment. Moreover, due to recurrent or chronic infections and/or hematopoietic stem cell transplantation, they receive prolonged or frequent treatment or prophylaxis with antibiotics and/or antifungals. The examples include antifungal prophylaxis in SCID patients and, after resolution of oral candidiasis, in CVID individuals (3,23,25-41).

Another important factor for developing diarrhea due to opportunistic pathogens is geographical location and the climate patients occupy. The studies reporting *Candida* and *Trichosporon* as etiology of diarrhea were primarily from Asian and African countries with relatively hot climate (28,30,32,33,41).

Altogether, minor differences between our results and those reported by some other studies could be attributed to these factors: 1) different sample size, 2) different antibiotic or antifungal regimens patients received, 3) different geographical locations and climate occupied by the patients, 4) some limitations in laboratory investigation of viral pathogens in our study, and 5) probably ignoring laboratory investigation of unusual organisms in some studies from countries in Europe and North America, since pathogenicity of these organisms has not yet been observed in these countries with different climate than locations occupied by some of our patients.

Accordingly, it is recommended that facultative or opportunistic pathogens to be taken into consideration in laboratory investigation of infectious etiology of diarrhea in PID, especially in the cases when no common pathogen is found, in patients who receive prophylaxis or prolonged or repeated treatment with antibiotics or antifungals, and in patients from relevant geographical locations.

Conflicts of interest statement

The authors declare that they have no conflict of interest.

References

1. Lindegren ML, Kobrynski L, Rasmussen SA, Moore CA, Grosse SD, Vanderford ML, Spira TJ, McDougal JS, Vogt RF, Jr., Hannon WH, et al. Applying public health strategies to primary immunodeficiency diseases: a potential approach to genetic disorders. MMWR Recomm Rep 2004; 53:1-29.
2. Agarwal S, Mayer L. Gastrointestinal manifestations in primary immune disorders. Inflamm Bowel Dis 2010; 16:703-711.
3. UpToDate. Aghamohammadi A, Zeinoddini A, Mohammadinejad P, Rezaei N. Gastrointestinal manifestations in primary immunodeficiency. In: UpToDate. Stiehm ER (Ed), UpToDate, Waltham, MA. (Accessed on April 14, 2015.).
4. Agarwal S, Mayer L. Diagnosis and treatment of gastrointestinal disorders in patients with primary immunodeficiency. Clin Gastroenterol Hepatol 2013; 11:1050-1063.
5. Aghamohammadi A, Mohammadinejad P, Abolhassani H, Mirminachi B, Movahedi M, Gharagozlou M, Parvaneh N, Zeiaee V, Mirsaeed-Ghazi B, Chavoushzadeh Z, et al. Primary immunodeficiency disorders in Iran: update and new insights from the third report of the national registry. J Clin Immunol 2014; 34:478-490.

6. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol 1999; 93:190-197.
7. Vanderhoof JA. Chronic diarrhea. Pediatr Rev 1998; 19:418-422.
8. Lai KK, Lamps LW. Enterocolitis in immunocompromised patients. Semin Diagn Pathol 2014; 31:176-191.
9. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency. clinical and immunological features of 248 patients. Clin Immunol 1999; 92:34-48.
10. McCabe RP. Gastrointestinal Manifestations of Non-AIDS Immunodeficiency. Curr Treat Options Gastroenterol 2002; 5:17-25.
11. Atarod L, Raissi A, Aghamohammadi A, Farhoudi A, Khodadad A, Moin M, Pourpak Z, Movahedi M, Charagozlou M, Rezaei N. A review of gastrointestinal disorders in patients with primary antibody immunodeficiencies during a 10-year period (1990-2000), in children hospital medical center. Iran J Allergy Asthma Immunol 2003; 2:75-79.
12. Oksenhendler E, Gerard L, Fieschi C, Malphettes M, Mouillot G, Jaussaud R, Viallard JF, Gardembas M, Galicier L, Schleinitz N, et al. Infections in 252 patients with common variable immunodeficiency. Clin Infect Dis 2008; 46:1547-1554.
13. Noel J, Cubitt D. Identification of astrovirus serotypes from children treated at the Hospitals for Sick Children, London 1981-93. Epidemiol Infect 1994; 113:153-159.
14. 14. Gallimore CI, Taylor C, Gennery AR, Cant AJ, Galloway A, Iturriza-Gomara M, Gray JJ. Environmental monitoring for gastroenteric viruses in a pediatric primary immunodeficiency unit. J Clin Microbiol 2006; 44:395-399.
15. Frange P, Touzot F, Debre M, Heritier S, Leruez-Ville M, Cros G, Rouzioux C, Blanche S, Fischer A, Avettand-Fenoel V. Prevalence and clinical impact of norovirus fecal shedding in children with inherited immune deficiencies. J Infect Dis 2012; 206:1269-1274.
16. Aghamohammadi A, Fiorini M, Moin M, Parvaneh N, Teimourian S, Yeganeh M, Goffi F, Kanegane H, Amirzargar AA, Pourpak Z, et al. Clinical, immunological and molecular characteristics of 37 Iranian patients with X-linked agammaglobulinemia. Int Arch Allergy Immunol 2006; 141:408-414.
17. Winkelstein JA, Marino MC, Lederman HM, Jones SM, Sullivan K, Burks AW, Conley ME, Cunningham-Rundles C, Ochs HD. X-linked agammaglobulinemia: report on a United States registry of 201 patients. Medicine 2006; 85:193-202.
18. Winkelstein JA, Marino MC, Ochs H, Fuleihan R, Scholl PR, Geha R, Stiehm ER, Conley ME. The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. Medicine 2003; 82:373-384.
19. Brett MM, Rodhouse JC, Donovan TJ, Tebbutt GM, Hutchinson DN. Detection of Clostridium perfringens and its enterotoxin in cases of sporadic diarrhoea. J Clin Pathol 1992; 45:609-611.
20. Mpamugo O, Donovan T, Brett MM. Enterotoxigenic Clostridium perfringens as a cause of sporadic cases of diarrhoea. J Med Microbiol 1995; 43:442-445.
21. Borriello SP, Larson HE, Welch AR, Barclay F, Stringer MF, Bartholomew BA. Enterotoxigenic Clostridium perfringens: a possible cause of antibiotic-associated diarrhoea. Lancet 1984; 1:305-307.
22. Modi N, Wilcox MH. Evidence for antibiotic induced Clostridium perfringens diarrhoea. J Clin Pathol 2001; 54:748-751.
23. Asha NJ, Tompkins D, Wilcox MH. Comparative analysis of prevalence, risk factors, and molecular epidemiology of antibiotic-associated diarrhea due to Clostridium difficile, Clostridium perfringens, and Staphylococcus aureus. J Clin Microbiol 2006; 44:2785-2791.
24. Skjelkvale R, Uemura T. Experimental Diarrhoea in human volunteers following oral administration of Clostridium perfringens enterotoxin. J Appl Bacteriol 1977; 43:281-286.
25. Adlard PA, Kirov SM, Sanderson K, Cox GE. Pseudomonas aeruginosa as a cause of infectious diarrhoea. Epidemiol Infect 1998; 121:237-241.
26. Kim SW, Peck KR, Jung SI, Kim YS, Kim S, Lee NY, Song JH. Pseudomonas aeruginosa as a potential cause of antibiotic-associated diarrhea. J Korean Med Sci 2001; 16:742-744.
27. Talwar P, Chakrabarti A, Chawla A, Mehta S, Walia BN, Kumar L, Chugh KS. Fungal diarrhoea: association of different fungi and seasonal variation in their incidence. Mycopathologia 1990; 110:101-105.
28. Enweani IB, Obi CL, Jokpeyibo M. Prevalence of Candida species in Nigerian children with diarrhoea. J Diarrhoeal Dis Res 1994; 12:133-135.
29. Jindal N, Arora R, Bhushan B, Arora S. A study of infective aetiology of chronic diarrhoea in children in Amritsar. J Indian Med Assoc 1995; 93:169-170.
30. Chaudhury A, Nath G, Shukla B, Panda S, Singh TB. Diarrhoea associated with Candida spp.: incidence and seasonal variation. J Diarrhoeal Dis Res 1996; 14:110-112.
31. Nkuo-Akenji TK, Ndip RN, Ntoko FA. The prevalence of Candida albicans-associated diarrhoea in Buea, South West Cameroon. Afr J Health Sci 2002; 9:153-157.
32. Simadibrata M, Tytgat GN, Yuwono V, Daldiyono, Lesmana LA, Syam AF, Ariawan I, Rani A. Microorganisms and parasites in chronic infective diarrhea. Acta Med Indones 2004; 36:211-214.
33. Banerjee P, Kaur R, Uppal B. Study of fungal isolates in patients with chronic diarrhea at a tertiary care hospital in north India. J Mycol Med 2013; 23:21-26.
34. Loy CE. Antibiotic-associated diarrhoea: an overlooked aetiology? Br J Biomed Sci 2005; 62:166-169.
35. Same-Ekobo A, Lohoue J, Mbassi A. (A clinical and biological study of parasitic and fungal diarrhea in immunosuppressed patients in an urban and suburban area of Yaounde). Sante 1997; 7:349-354.
36. 36. Punpoowong B, Viriyavejakul P, Riganti M, Pongponaratn E, Chaisri U, Maneerat Y. Opportunistic protozoa in stool samples from HIV-infected patients. Southeast Asian J Trop Med Public Health 1998; 29:31-34.
37. Kane JG, Chretien JH, Garagusi VF. Diarrhoea caused by Candida. Lancet 1976; 1:335-336.
38. Gupta TP, Ehrinpreis MN. Candida-associated diarrhea in hospitalized patients. Gastroenterology 1990; 98:780-785.
39. Haupt HM, Merz WG, Beschorner WE, Vaughan WP, Saral R. Colonization and infection with Trichosporon species in the immunosuppressed host. J Infect Dis 1983; 147:199-203.
40. Agirbasli H, Bilgen H, Ozcan SK, Otlu B, Sinik G, Cerikcioglu N, Durmaz R, Can E, Yalman N, Gedikoglu G, et al. Two possible cases of Trichosporon infections in bone-marrow-transplanted children: the first case of T. japonicum isolated from clinical specimens. Jpn J Infect Dis 2008; 61:130-132.
41. Colombo AL, Padovan AC, Chaves GM. Current knowledge of Trichosporon spp. and Trichosporonosis. Clin Microbiol Rev 2011;24:682-700.

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Delayed hypersensitivity to new oral anticoagulants. Demonstration of cross reactivity for the drug category and definition of non-irritant concentrations for patch tests

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Keywords
direct trombin inhibitors; delayed hypersensitivity; edoxaban; atrial fibrillation; patch test

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Summary
The current therapy with direct trombin inhibitors (DTI) is indicated for the prevention of stroke in non-valvular atrial fibrillation. Side effects are reported, particularly skin hypersensitivity, for this whole category of drugs as well as for other modern antiplatelet and anticoagulant drugs.
For their clinical features, these reactions appear as delayed T-cell mediated drug hypersensitivity, but at present there are no diagnostic methods of investigation. We reported a case of delayed skin reaction to edoxaban and we found the non-irritant concentration for patch test in the whole category of drugs.
The patch test resulted positive for edoxaban. A successive challenge with alternative DTIs and/or a switch to warfarin is proposed as alternative therapy.

Introduction

The new oral anticoagulants, Direct Thrombin Inhibitors (DTIs) or Non-vitamin K Oral AntiCoagulants (NOACs) are a class of anticoagulant drugs indicated for the prevention of strokes and systemic embolism in non-valvular atrial fibrillation (1). This class of anticoagulant drugs has a direct effect on the Factor X of the coagulation cascade, and doesn't require the use of antithrombin as mediator. In Italy there are four molecules approved by the Italian Drug Agency (AIFA): rivaroxaban, apixaban, edoxaban, dabigatran. Dabigatran acts selectively by inhibiting thrombin. Edoxaban is a direct inhibitor of factor Xa. The clinical use of these drugs is similar to the use of the dated vitamin K antagonists (warfarin and acenocoumarol, also called AVK), which actually have an effect on different levels of the coagulation cascade, acting on the synthesis of various coagulation factors (II, VII, IX, X) in the liver.
According to the ESC guidelines (European Society of Cardiology), non-vitamin K oral anticoagulants (NOACs) are indicated for patients: (2) with non-valvular atrial fibrillation (FANV) lasting ≥ 48h, or when the duration of atrial fibrillation is unknown. Oral anticoagulant treatment (e.g. vitamin K antagonists with INR 2-3 or NOACs) is recommended for ≥ 3 weeks before and for ≥ 4 weeks after cardioversion.
In patients with risk factors for stroke or recurrent atrial fibrillation (FANV), oral anticoagulant treatment, either with AVK (INR 2-3) or new oral anticoagulants, should be continued chronically, regardless of the apparent maintenance of sinus rhythm after cardioversion.

Post-marketing observations have shown side effects (3) for the whole category of these drugs, especially skin hypersensitivity. Dermatitis have been reported, with varying frequency: for edoxaban, a skin reaction is an adverse effect reported as common. Rivaroxaban (4-13) has shown a similar dermatitis frequency; as for apixaban, the reaction is uncommon (14), while it is rare in the case of dabigatran (15-19) (**figure 1**).
These reactions appear for their clinical feature as a delayed T-cell mediated drug hypersensitivity, but at present there are no diagnostic methods of investigation.
For this reason, we report a clinical case demonstration of the presence of T-cell mediated sensitization caused by these drugs; given this case, we propose a diagnostic protocol and choice of alternative NAOs.

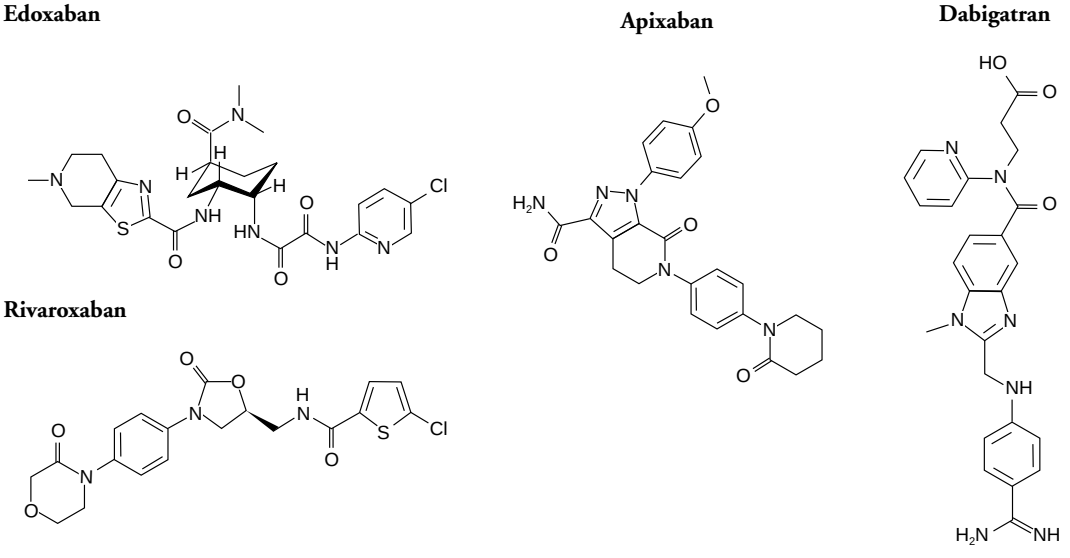
Clinical case

The patient is a seventy-year-old woman discharged from department of Internal Medicine with the following diagnosis: heart failure in atrial fibrillation; hypertensive heart disease with hypokinetic-dilatative evolution and mitral valvular prolapse, aneurysm of the interventricular septum. The patient has been treated with: furosemide 25 mg, kanrenoatus 2 mg, ramipril 5 mg, bisoprolol 2.5 mg, allopurinol 150 mg, enoxaparin 8000 IU 1 fl sc twice a day. During the recovery the patient has started therapy with edoxaban, 60 mg 1 cp daily.
Objectively, the patient appeared to be dyspneic even after mild efforts, and presented malleolar oedema. Ten days after the start of the edoxaban therapy, she showed symptoms of prurigo and widespread erythematous lesions. These lesions were attributed to edoxaban, for which the skin reaction is an adverse effect reported as common. Therefore, the patient underwent an allergological examination and skin tests for calciparin, enoxaparin, nadroparin, which resulted negative.
Consequently, the treatment with edoxaban was suspended, while the treatment with enoxaparin alone was continued. The symptoms gradually resolved at home. The possible adverse reaction to edoxaban as "culprit drug" was reported to the Internal Pharmacy.
Considering the available data about possible adverse reactions, the drug to be preferred seemed to be dabigatran.

Material and methods

A galenic preparation was set up in the Hospital Pharmacy: rivaroxaban-Xarelto®, 20 mg whole tablets crushed in a mortar and mixed with vaseline at 10% and 30%; the same preparation was adopted for: dabigatran-Pradaxa® 150 mg tablets, at 10 and 30% vaseline; apixaban-eliquis® 5 mg tablets at 10 and 30% vaseline; edoxaban-Lixiana® 60 mg tablets at 10 and 30% vaseline.
Six healthy controls tested negative with identical preparation. The patch was negative at 72 h, but after five days it tested positive for dabigatran-pradaxa® at 10% +, and at 30% ++, and for edoxaban-Lixiana® + at 30%. The patient then performed a gradual challenge procedure with apixaban-eliquis® (negative patch test) 2.5 mg on the first day, tolerated, then after 24 h in two administrations, tolerated as well. Unluckily, the drug

Figure 1 *- Chemical structure of direct trombin inhibitors.*



was tolerated only for seven days; after that, symptoms of widespread eczematous dermatitis started to appear again.
Hence the administration of apixaban was halted, and the patient was treated with an antihistamine and oral steroid; subsequently she showed remission of symptoms in seven days; she underwent simultaneous reintroduction of enoxaparin.
The following cardiological choice was to switch to an oral anticoagulant therapy with warfarin, which was finally tolerated by the patient.

Discussion

The new oral anticoagulants present relatively common skin reactions according to post-marketing surveillance data. The most common reactions are of the erythematous eczematous type. On the other hand, various and more serious clinical features are reported in literature: leukocytoclastic vasculitis, drug induced hypersensitivity syndrome (DIHS) (7), drug induced drug reaction with eosinophilia and systemic symptoms (DRESS) (8).
Eczematous dermatitis seems to be caused by T cells mediated immune reaction. The patch test is therefore an appropriate method for the diagnosis of such reactions. The exposed case highlights for the first time the presence of a specific T-cells delayed type hypersensitivity to these drugs. The concentration of such drugs crushed as whole tablets in a mortar and mixed in vaseline at 30% appears to be non-irritant on the tested healthy subjects and can be used for diagnosis. An even more delayed reading of the test, after a few days, appears significant. We also established the non-irritant concentration for patch test and we suggest a late lecture of the test at five days. The presence of allergic cross reactivity among the category of drugs is also demonstrated.
The challenge with a new alternative oral anticoagulant or the switch therapy to warfarin may be the subsequent therapeutic choice.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Ogawa S, Shinohara Y, Kanmuri K. Safety and efficacy of the oral direct factor xa inhibitor apixaban in Japanese patients with non-valvular atrial fibrillation. The ARISTOTLE-Jstudy. Circ J 2011; 75(8):1852-1859. Epub 2011 Jun 14.
2. De Caterina R, Husted S, Wallentin L, Andreotti F, Arnesen H, Bachmann F, Baigent C, Collet JP, Halvorsen S, Huber K, Jespersen J, Kristensen SD, Lip GY, Morais J, Rasmussen LH, Ricci F, Sibbing D, Siegbahn A, Storey RF, Ten Berg J, Verheugt FW, Weitz JI. Oral anticoagulants in coronary heart disease (Section IV). Position paper of the ESC Working Group on Thrombosis - Task Force on Anticoagulants in Heart Disease. Thromb Haemost 2016; 115(4):685-711. doi: 10.1160/TH15-09-0703. Epub 2016 Mar 8. Review.
3. Hofmeier KS. Hypersensitivity reactions to modern antiplatelet and anticoagulant drugs. Allergo J Int 2015; 24(2):58-66. Epub 2015 Mar 14.
4. Yates J, Choudhry M, Keys G. A case report describing a suspected rivaroxaban hypersensitivity reaction in a surgical patient. J Clin Pharm Ther 2013; 38(2):159-161. doi: 10.1111/jcpt.12013. Epub 2012 Nov 20.
5. Christopoulou EC, Filippatos TD, Elisaf MS. Non-hemorrhage-related adverse effects of rivaroxaban. Arch Med Sci Atheroscler Dis 2017; 2:e108-e112. doi: 10.5114/amsad.2017.72533. eCollection 2017.
6. Rudd KM, Panneerselvam N, Patel A. Rash associated with rivaroxaban use.Am J Health Syst Pharm 2018; 75(6):347-349. doi: 10.2146/ajhp160985. Epub 2018 Jan 18.
7. Chiasson CO, Canneva A, Roy FO, Doré M. Rivaroxaban Induced Hypersensitivity Syndrome. Can J Hosp Pharm 2017; 70(4):301-304. Epub 2017 Aug 31.
8. Radu C, Barnig C, de Blay F. Rivaroxaban-Induced Drug Reaction With Eosinophilia and Systemic Symptoms. JInvestigAllergol Clin Immunol 2016; 26(2):124-126. doi: 10.18176/jiaci.0027.
9. Chaaya G, Jaller-Char J, Ghaffar E, Castiglioni A.Rivaroxaban-induced leukocytoclastic vasculitis: A challenging rash. Ann Allergy Asthma Immunol 2016; 116(6):577-8. doi: 10.1016/j.anai.2016.03.012. Epub 2016 Mar 24.
10. Vernon HM, Nielsen AK, O'Bryan EC. Hypersensitivity reaction after administration of rivaroxaban (Xarelto). Am J Emerg Med 2016; 34(7):1325.e1-2. doi: 10.1016/j.ajem.2015.12.021. Epub 2015 Dec 17.
11. Snyder DJ, Matusik FB. Rivaroxaban-induced serum sickness after total knee arthroplasty. Am J Health Syst Pharm 2015; 72(18):1567-1571. doi: 10.2146/ajhp140549.
12. Barrett P, Vuppalanchi R, Masuoka H, Chalasani N. Severe drug-induced skin and liver injury from rivaroxaban. Dig Dis Sci 2015; 60(6):1856-8. doi: 10.1007/s10620-014-3504-9. Epub 2015 Jan 6. Erratum in: Dig Dis Sci. 2015 Jul;60(7):2210.
13. Altin C, Ozturkeri OA, Gezmis E, Askin U. Angioedema due to the new oral anticoagulant rivaroxaban. Ann Card Anaesth 2014; 17(2):173-4.doi: 10.4103/0971-9784.129888.
14. Veliyev V, Özmen İ, Yaşar S, Gürsoy E, Köklü M, Çelik M. A rare side effect seen due to the use of apixaban: Palmoplantar psoriasiform drug eruption. Anatol J Cardiol 2016; 16(3):213-215. doi: 10.14744/AnatolJCardiol.2016.6774.
15. Eid TJ, Shah SA. Dabigatran-induced rash. Am J Health Syst Pharm. 2011; 68(16):1489-1490. doi: 10.2146/ajhp110088.
16. To K, Reynolds C, SpinlerSA. Rash associated with dabigatran etexilate. Pharmacotherapy 2013; 33(3):e23-7. doi: 10.1002/phar.1203. Epub 2013 Feb 11.
17. Lyu C, He Y, Wei Z, Zeng W. Zhonghua Xin Xue Guan Bing Za Zhi. Dabigatran induced drug eruption: a case report. 2015; 43(10):918. Chinese.
18. Cakmak MA, Sahin S, Cinar N, KarsidagS. Adverse skin reaction caused by dabigatran. Eur Rev Med Pharmacol Sci 2014; 18(18):2595.
19. An J, Garje R, Wanat KA, Leone. Dabigatran-related leukocytoclastic vasculitis. JP BMJ Case Rep 2017; 2017. pii: bcr2016217423. doi: 10.1136/bcr-2016-217423.

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Allergic bronchopulmonary aspergillosis screening in bronchiectasis: is there always a precise answer to a clear question?

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Key words

bronchiectasis; allergic bronchopulmonary aspergillosis; severe asthma with fungal sensitisation; Aspergillus-associated syndromes; bronchiectasis screening

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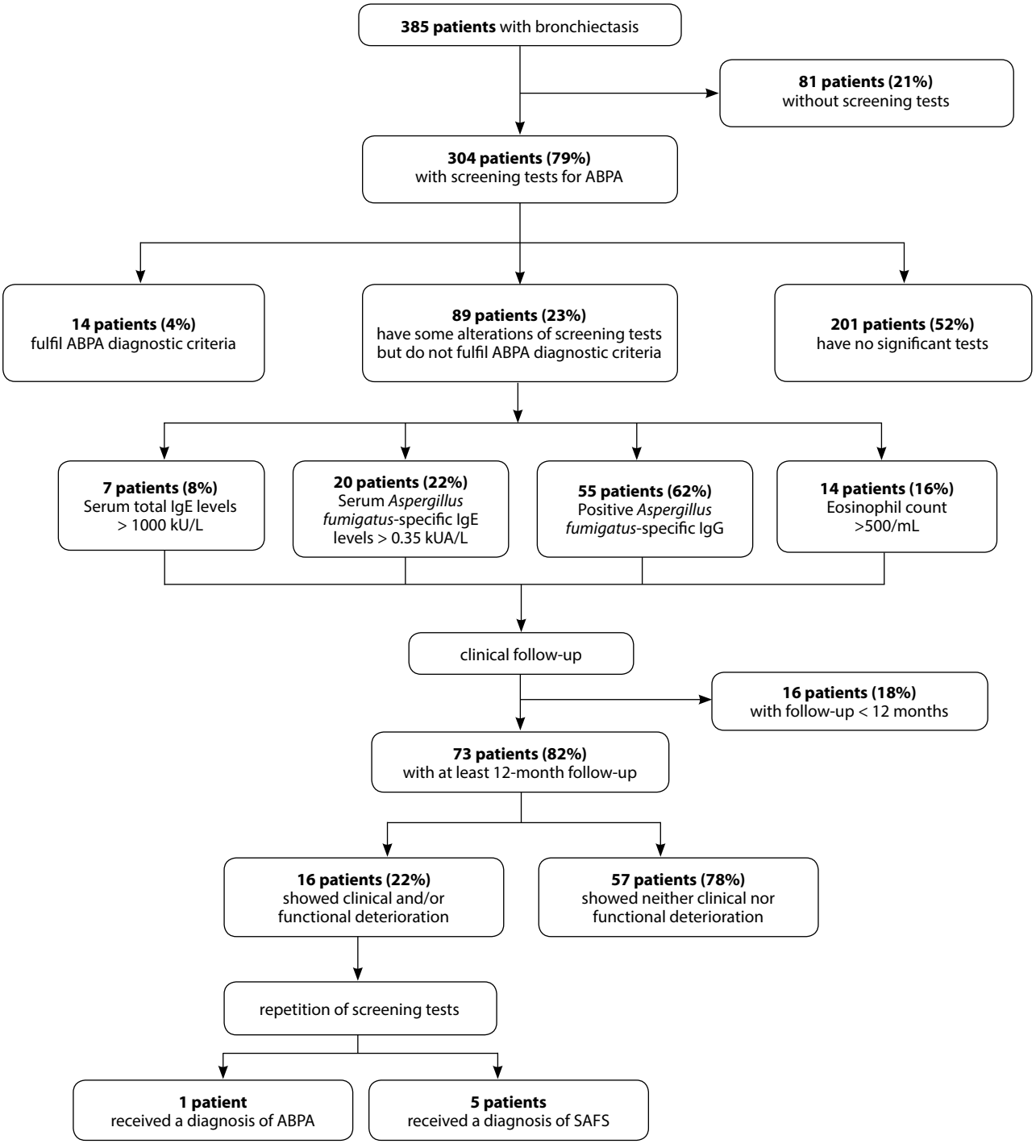
To the Editor

The most recently published guidelines about the management of adult bronchiectasis suggest screening all patients for allergic bronchopulmonary aspergillosis (ABPA), since this is a potentially treatable disease and modifiable cause of bronchiectasis (1). Tests recommended include total serum IgE, specific IgG to *Aspergillus* and specific IgE to *Aspergillus* or, as an alternative, skin prick tests to *Aspergillus*.

ABPA prevalence in adults with bronchiectasis is low, and varies between 1 and 11% in different cohorts (2,3). However, in some cases, tests for ABPA might be altered but not consistent with a diagnosis of ABPA. No recommendations are available about management of these patients with only some nonspecific serological alterations suggestive of ABPA, and about their risk to develop ABPA or other *Aspergillus*-associated syndromes, such as severe asthma with fungal sensitisation (SAFS) (4), during follow-up.

To answer these questions, we retrospectively reviewed our prospectively-collected cohort of consecutive adult patients with non-cystic fibrosis bronchiectasis attending the outpatient clinic at the San Gerardo Hospital, Monza, Italy, from January 1st, 2013 to December 1st, 2016 (Institutional Review Board approval n. 234, September 2013). To define ABPA we used the diagnostic criteria proposed by the ABPA Working Group ISHAM 2013 (5). After screening visit, all patients were offered a clinical and functional follow-up at our outpatient clinic. Clinical deterioration was established by the attending physicians,

Figure 1 *- Flow chart of study population.*



ABPA, allergic bronchopulmonary aspergillosis; SAFS, severe asthma with fungal sensitisation.

according to the following parameters: onset or worsening of respiratory symptoms, such as cough, dyspnea and sputum production, or pulmonary exacerbations ≥ 2/year. Functional deterioration was defined as Forced Expiratory Volume in the 1st second (FEV1) and/or Forced Vital Capacity (FVC) > 10% reduction compared to the prior test.

Out of 385 patients in our cohort, the prevalence of ABPA was 4%, consistent with the prior literature (2-3) (**figure 1**). Almost one quarter (89, 23%) of our patients presented, at the time of first evaluation, one or more alterations of ABPA testing (**figure 1**). Eighteen (20%) patients showed multiple contemporary alterations of screening exams.

Out of 73 patients with nonspecific ABPA screening alterations at baseline and at least a 12-month clinical follow-up (median follow-up time 25 months), the majority (57 patients, 78%) showed neither clinical nor functional deterioration and none of them developed *Aspergillus*-associated syndromes, while a minority of patients (16 - 22%) repeated screening tests because of clinical and/or functional deterioration. In these patients the repetition of screening tests, together with functional and clinical evolution, allowed to make a diagnosis of ABPA in 1 case and SAFS in 5 cases.

In conclusion, only a minority of bronchiectasis patients (4%) were diagnosed with ABPA at baseline, while nonspecific alterations of screening tests not consistent with a diagnosis of ABPA were common (almost one quarter of cases in our cohort). In this specific group, 22% of patients showed clinical and/or functional deterioration during follow-up and almost 8% received a diagnosis of ABPA or SAFS. Therefore, a non-negligible proportion of patients with nonspecific alterations of screening tests at baseline may develop *Aspergillus*-associated syndromes during follow-up. Such condition should be suspected in particular in the presence of clinical and/or functional deterioration. Further studies on multi-centric cohorts and longer follow-up periods are needed to confirm our observations.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Polverino E, Goeminne PC, McDonnell MJ, Aliberti S, Marshall SE, Loebinger M, et al. European Respiratory Society guidelines for the management of adult bronchiectasis. Eur Respir J 2017; 9:50(3).
2. De Soyza A, Aliberti S. Bronchiectasis and Aspergillus: How are they linked? Med Mycol 2017; 55(1):69-81.
3. Suarez-Cuartin G, Chalmers JD, Sibila O. Diagnostic challenges of bronchiectasis. Respir Med 2016; 116:70-77.
4. Agarwal R. Severe asthma with fungal sensitization. Curr Allergy Asthma Rep 2011; 11:403-413.
5. Agarwal R, Chakrabarti A, Shah A, Gupta D, Meis JF, Guleria R, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. Clin Exp Allergy 2013; 43(8):850-873.

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Allergy in adolescent population (14-18 years) living in Campania region (southern Italy). A multicenter study

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Key words

adolescents; allergy; allergic rhinitis; allergic sensitization; bronchial asthma; Campania region; elderly; hypersensitivity

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Summary

Adolescents (Ad) constitute a difficult to manage population among individuals suffering from asthma. The aim of our study was to assess the prevalence, clinical characteristics and age of onset of allergic sensitization and clinical symptoms in a sample of atopic Ad living in the Campania region (Southern Italy). Sixteen Allergy units or Centers belonging to the Italian Association of Hospital and Territorial Allergologists (AAIITO, Campania region) participated in this cross-sectional study. A case report form (CRF) was specifically designed for this study and commercial allergen extracts used for screening SPTs were provided by ALK-Abelló Group (Milan, Italy). A total of 443 patients were examined (females, F 220, 49.6%; males, M 223, 50.3%). Dust mites represent the most common sensitizing agents in allergic Ad living in Campania region (Dermatoph. pteronyssinus 67.4% and Dermatoph. farinae 66.5%), followed by Parietaria (58.9%), grasses (45.8%), Artemisia vulgaris (16.7%), Olea Europaea (32.2%), dog dander (17.1%), cat dander (20.0%), Alternaria alternata (8.1%), Cupressus sempervirens (4.9%), Betula pendula (4.7%), other allergens (19.4%). An interesting comparison has been made between clinical data of our Ad with data of elderly patients (E). The role of allergic sensitization is significantly higher in Ad compared to E. Dermatophagoides pteronyssinus is the first sensitizing allergen in Ad and the last in E. Parietaria constitutes the first sensitizing pollen both in Ad and E, the percentage of sensitization is higher in Ad. Another important difference is the higher prevalence of asthma (As), as only symptom, in E compared to Ad (19.7% versus 7.6%).

In conclusion, our findings confirm the high prevalence and clinical significance of airway allergic sensitization in the adolescents living in Campania region.

To the Editor

It is widely recognized that Ad constitute a difficult to manage population among individuals suffering from asthma. In fact, asthmatic Ad may experience a period of physical and psychosocial changes that affect their health and well-being. Overall, Ad with asthma are at increased risk for asthma morbidity and death. Increased rates of depression and anxiety, for the Ad and their caregivers, can lead to non-adherence to their medical regimens, poor symptom control and poor treatment outcomes (1). Contextual factors, such as race, ethnicity, and personal characteristics (particularly cigarette smoke or the use of narcotics), affect the prevalence, morbidity, and mortality for the Ad with asthma. These factors also affect the transition process for Ad entering adult medical care (2). As a consequence of these factors, asthma during adolescence impairs health-related quality of life, especially if the asthma is uncontrolled (3). Based on this background, the aim of our study was to assess the prevalence, clinical characteristics and age of onset of allergic sensitization and clinical symptoms in a sample of atopic Ad living in Campania region (southern Italy).

Sixteen Allergy units or Centres belonging to the Italian Association of Hospital and Territorial Allergologists (AAIITO, Campania region), uniformly distributed over the whole territory of Campania region (13,595 Km^2, 5,833,332 inhabitants at 30 November 2014) participated in this cross-sectional study. The same protocol was shared by all participating centers; each centre collected the results of allergy consultations of consecutive outpatients, aged 14 - 18 years, referred for suspected or current respiratory allergy (asthma and/or rhinitis). Patient enrollment started on January 1 and ended on June 30, 2017.

A case report form (CRF) specifically designed for this study was completed during the screening consultation of each patient. The standardized form reported: demographic data, type and duration of respiratory symptoms, pet ownership, results of skin prick tests (SPTs), age of onset of respiratory symptoms. The diagnosis of respiratory allergy has been carried out according to the International Guidelines (4,5).

The commercial allergen extracts used for screening SPTs were provided by ALK-Abellò Group (Milan, Italy). A standard panel of allergens was used, including *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae, Alternaria alternata, Cladosporium herbarum,* cat and dog dander, *Parietaria,* grass pollen mix, *Artemisia vulgaris, Olea europaea, Betula pendula, Cupressus sempervirens* and *Corylus avellana*. This allergen panel covers the main causative agents of respiratory allergy in Campania region. Positive (10 mg/ml histamine HCl) and negative (saline solution in glycerine-phenol solution) controls were used. SPTs were performed and interpreted according to International Guidelines (6); results were read after 15 minutes and expressed as the mean of the major wheal diameter plus its orthogonal. A skin reaction of 3 mm or greater was considered positive. Wheal profiles were outlined using a fine-point marking pen and transferred by adhesive tape onto the patient's case report form.

Patients with chronic or dysmetabolic diseases, severe cutaneous disorders, negative skin reaction to histamine, or undergoing treatment with drugs interfering with skin response were excluded from the study (7,8).

A total of 443 patients were examined (females, F 220, 49.6%; males, M 223, 50.3%). Three hundred and fifty subjects (76.7%) had positive SPTs to at least one allergen and were diagnosed with respiratory allergy, the remaining 103 (23.2%) were SPTs-negative. The prevalence of allergic sensitization

***Figure 1** - Prevalence of allergic sensitization to perennial/seasonal allergens in adolescents living in Campania region.*

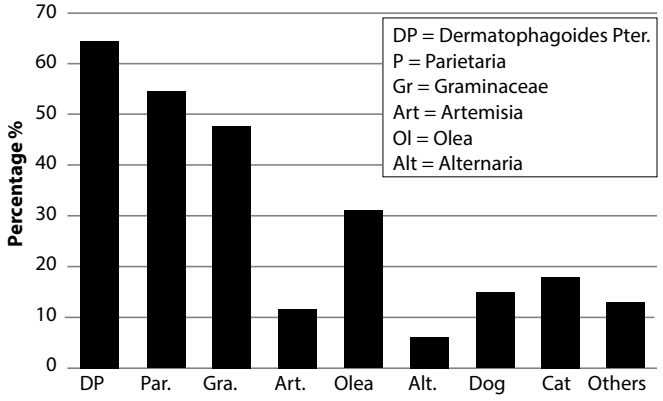
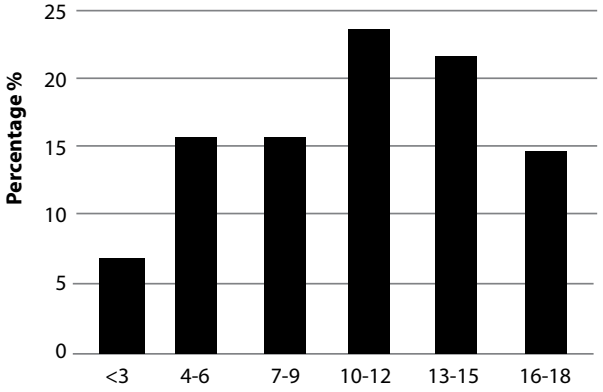


***Figure 2** - Age of onset of respiratory symptoms in enrolled adolescents living in Campania region.*



was almost identical in both sexes (50.3% M and 49.6% F). Dust mites represent the most common sensitizing agents in allergic adolescents living in Campania region (*Dermatoph. pteronyssinus* 67.4% and *Dermatoph. farinae* 66.5%), followed by *Parietaria* (58.9%), grasses (45.8%), *Artemisia vulgaris* (16.7%), *Olea europaea* (32.2%), dog dander (17.1%), cat dander (20.0%), *Alternaria alternata* (8.1%), *Cupressus sempervirens* (4.9%), *Betula pendula* (4.7%), other allergens (19.4%) (**figure 1**).

These data are in agreement with previous reports on children and adults living in Campania region and Naples area, where the most common sensitizing agents were dust mites followed by *Parietaria*, Grass pollen and *Olea europaea* (9,10). Most of our Ad shows a poly in comparison to mono pattern of allergic sensitization, and this finding is confirmed also by other authors (11,12). As regards clinical symptoms, 64.5% of our patients reported exclusively rhinitis (R), 7.6% only asthma (As), 32.2% R + As, and finally 55.3% conjunctivitis. Nasal symptoms are present in the totality (100%), whereas bronchial symptoms in 40.7% of our adolescents.

The age of onset of clinical symptoms in our patients is shown in **figure 2**. An interesting comparison can be made between Ad and E patients living in Campania region, because data are comparable being produced by the same working group (**figure 3**).

The number of patients is comparable in both ages (443 Ad and 462 E), sex rate is similar in Ad (F 49.6%, M 50.3%), a significant prevalence of F can be observed in E (F 62.9%, M 37.0%). In fact, adolescence is the age of change in the prevalence of respiratory allergy, which is higher in M during paediatric age and in F during adulthood. A positive SPT for at least one allergen was found in 76.7% of A and in 46.5% of E. The prevalence of allergic sensitization was almost identical in both sexes in Ad (M 50.1% and F 49.9%), whereas female sex is prevalent, as expected, in E (F 60.7%, M 39.3%). Dust mites constitute the most frequent sensitizing agent in Ad, whereas pollens are involved in respiratory allergy in E. In order of frequency, R, R + As and As represent the most common clinical symptoms both in Ad and in E.

Figure 3 clearly shows that the role of allergic sensitization to the four most common allergens in Campania region is significantly higher in Ad compared to E. In particular, *Dermatophagoides pteronyssinus* is the first sensitizing allergen in Ad and the last in E according to its early contact with airways. *Parietaria* constitutes the first sensitizing pollen both in Ad and E, the percentage of sensitization is higher in Ad. Another important difference showed by **figure 3** is the higher prevalence of As, as only symptom, in E compared to Ad (19.7% versus 7.6%). The higher prevalence of As in E is likely the consequence of a longer exposure to environmental or professional risk factors.

In conclusion, our data show that the prevalence and clinical importance of airway allergic sensitization in the Ad living in Campania region is very relevant. Our findings confirm that allergic sensitization in adolescence constitutes a turning point between the peculiar characteristics of the child and those of the adult. Present data on Ad integrate our knowledge on allergic population living in Campania region derived from previous studies (13-15).

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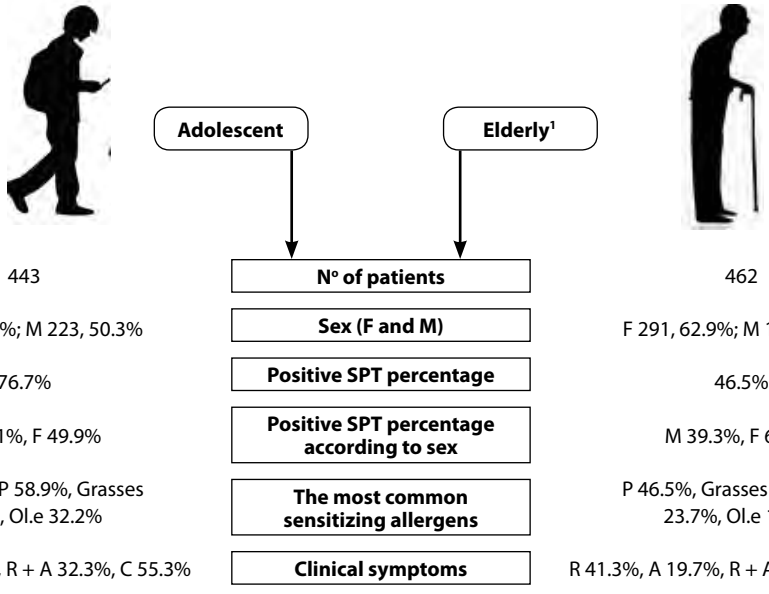
Conflict of interest and financial resources

All authors declare that they have no conflict of interest.

Figure 3 *- Comparative data of adolescent and elderly patients living in Campania region and suffering from suspected allergic airway diseases.*



F = female; M = male; DP = Dermatophagoides pteronyssinus; P = Parietaria; Ol. e = Olea europaea; R = Rhinitis; A = Asthma; C = Conjuctivitis; SPT = Skin Prick Test.
[1]Liccardi G. et al., Eur Ann Allergy Clin Immunol 2016; 48: 156-160 (15).

References

1. Bitsko MJ, Everhart RS, Rubin BK. The adolescent with asthma. Paediatr Respir Rev 2014; 15:146-153.
2. Fleming L, Murray C, Bansal AT, Hashimoto S, Bisgaard H, Bush A. The burden of severe asthma in childhood and adolescence: results from the paediatric U-BIOPRED cohorts. Eur Respir J 2015; 46:1322-1333.
3. Jonsson M, Bergstrom A, Egmar AC, Hedlin G, Lind T, Kull I. Asthma during adolescence impairs health-related quality of life. J Allergy Clin Immunol Pract 2016; 4:144-146.
4. Bousquet J and the ARIA Workshop Group. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001; 108:S147-S336.
5. Global Initiative for Asthma.http://ginasthma.com
6. Bousquet J, Heinzerling L, Bachert C, Papadopoulos NG, Bousquet PJ, Burney PG et al. Global Allergy and Asthma European Network; Allergic Rhinitis and its Impact on Asthma. Practical guide to skin prick tests in allergy to aeroallergens. Allergy 2012; 67:18-24.
7. Bousquet J, Michel FB. Precision of prick and puncture tests. J Allergy Clin Immunol 1992; 90:870-872.
8. Wever AMJ, Wever-Hess J. Testing for inhalant allergy in asthma. Clin Exp Allergy 1993; 23:976-981.
9. Liccardi G, Visone A, Russo M, Saggese M, D'Amato M, D'Amato G: Parietaria pollinosis: clinical and epidemiological aspects. Allergy Asthma Proc 1996; 17:23-29.
10. Liccardi G, Russo M, Piccolo A, Lobefalo G, Salzillo A, D'Amato M, D'Amato G: The perennial pattern of clinical symptoms in children monosensitized to Olea europaea pollen allergens in comparison with subjects with Parietaria and Gramineae pollinosis. Allergy Asthma Proc 1997; 18:99-105.
11. Anto JM, Bousquet J, Adkis M, Auffray C, Keil T, Momas I et al. Mechanisms of the development of allergy (MeDALL): Introducing novel concepts in allergy phenotypes. J Allergy Clin Immunol 2017; 139:388-399.
12. Schou Nielsen J, Meteran H, Ulrik CS, Porsbjerg C, Backer V. Natural history of skin prick test reactivity: A 20-year prospective study of a random population sample of children and adolescents. Ann Allergy Asthma Immunol. 2017; 119: 184-188.
13. Liccardi G, Baldi G, Ciccarelli A, Cutajar M, D'Amato M, Gargano D, Giannattasio D, Leone G, Lo Schiavo M, Madonna F, Montera C, Pio A, Russo M, Salzillo A, Stanziola A, D'Amato G. On behalf of Italian Association of Hospital and Territorial Allergologists (AAITO, Campania District, Southern Italy). Sensitization to rodents (mouse/rat) in urban atopic populations without occupational exposure living in Campania District (Southern Italy). A multicenter study. Multidiscip Respir Med 2013; 8:30.
14. Liccardi G, Baldi G, Ciccarelli A, Cutajar M, D'Amato M, Gargano D , Giannattasio D, Leone G, Lo Schiavo M, Madonna F, Montera C, Piccolo A, Pio A, Russo M, Stanziola A, D'Amato G. On behalf of Italian Association of Hospital and Territorial Allergologists (AAITO - Campania District, Southern Italy). Sensitization to cockroach allergens in the urban atopic populations living in Campania district (southern Italy). A multicenter study. Eur Ann Allergy Clin Immunol 2014; 46: 12-16.
15. Liccardi G, Baldi G, Berra A, Ciccarelli A, Cutajar M, D'Amato M et al. Italian Association of Hospital and Territorial Allergologists (AAIITO, Campania region). Allergy in urban elderly population living in Campania region (Southern Italy). A multicenter study. Eur Ann Allergy Clin Immunol 2016; 48:156-160.