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Severe asthma in adolescents and adults: a national, multicenter registry in real life

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Summary
The number of patients with uncontrolled asthma is growing especially in young people. Although current therapies improve the disease management, the heterogeneity of clinical outcomes results in patients whose asthma is refractory to standard therapies. To understand not responsive phenotypes, we instituted a web-registry aimed to collect real life data of adolescent and adult patients.
One-hundred and five Italian medical Centers are part of the network. Participants above 14 years and affected by severe asthma will be included in the study. Demographic and clinical data will be collected for 5 years on a dedicated electronic database.
For the first time in Italy, our study will provide information on epidemiological, clinical and therapeutic aspects related to the natural course of the disease, filling the gap between adolescents and adults.

Introduction

Asthma is a chronic heterogeneous respiratory obstructive airways disease characterized by different clinical phenotypes resulting from complex interaction between environmental and genetic factors (1). Although asthma remains one of the most prevalent diseases worldwide, with constantly growing numbers especially in pediatric age, it is often underdiagnosed and not properly treated. The results are a late diagnosis, low treatment efficacy (2), a reduced life expectancy accompanied by frequent hospitalizations and, consequently, high costs for the Health Care System (3-5).
For these reasons, in the past two decades the Global Initiative for Asthma (GINA) (6) developed a network of individuals, organizations, and public health officials to deliver the best care

to patients with asthma. However, due to asthma's multiple phenotypes, individuals can vary their response to the standard therapy, developing severe respiratory obstructive crises with a greater risk of *near fatal asthma* and mortality (7). To address the needing of the best approach to the management of that subgroup of patients, the American Thoracic Society and the European Respiratory Society (ATS/ERS) published recommendations and guidelines on the evaluation and treatment of severe asthma (SA) in children and adults (8). According to ATS/ERS, patients with SA require treatment with high doses of inhaled corticosteroids (ICS) plus a second controller as a long-acting β2-agonist, leukotriene modifier or theophylline and/or continuous or near continuous systemic corticosteroids (CS) (6,9-11).
In case of unsatisfactory control of asthma, the availability of biologic therapies enables addressing the patients to the most appropriate treatment and reducing therapeutics dropouts.
These treatments offer an improvement in the management of the disease, but the heterogeneity of clinical outcomes and phenotypes suggests that many subjects could be difficult to treat and drugs could be not efficacious, especially in children (12-14).
To face up this problem, the Society of Pediatric Respiratory Diseases (SIMRI) developed, for the first time in Italy, a registry for the collection of pediatric cases of SA with the aim to identify risks factors, clinical phenotypes and develop a *customized therapy* (15). Nevertheless, the SIMRI-registry results have two limitations: i) adolescents are not admitted to pediatric medical units and for this reason they are not recorded in the registry; ii) it is impossible to evaluate how the disease progresses and evolves from adolescence to adulthood. In turn, the Italian registry on severe/uncontrolled asthma has recently provided first results concerning adult patients exclusively (16).
In this context, the creation of a single registry to join pediatric and adult cases of SA is fundamental. The partnership of the Italian Association of Hospital Allergists and Immunologists (AAIITO) Italian Association of Hospital Pulmonologists (AIPO) proposed the institution of the Italian Registry on Severe Asthma (IRSA), aimed to collect data of *real life* in adolescents and adult patients. The IRSA will give the opportunity to follow the natural course of the disease, improving knowledge on the evolution of asthma phenotypes not responsive to the standard therapy, contributing to a more appropriate management of the patient and a more targeted design of future clinical studies.

Materials and methods

Study design

The IRSA is an ongoing observational, non-interventional, transversal and/or retrospective multicenter study of 5-years duration, involving 4.800 patients and 105 Italian Centers and local hospitals specialized in pulmonary and allergic diseases, planned thanks to the co-operations of AIPO Centro Studi (**figure 1**). The primary study outcome is to collect data of adolescents and adults affected by SA, in a "*real life*" setting, in order to: i) identify risks factors, ii) analyze multiple clinical phenotypes, and iii) understand the epidemiological, clinical and therapeutic aspects related to the pathology. To complete our results, a secondary outcome is carried out to determine etiological and clinical features of subjects with *near fatal* episodes of asthma.
Patients affected by SA are enrolled by the Unit of Allergy and Pulmonology of participating centers, at first admission (or retrospective) and followed-up every 12 months for the entire duration of the study (**figure 2**).
At the first visit, patients are asked to accept written informed consent, except for young people where parents or legal representatives have the responsibility to approve the participation to the study. The Investigator has to withdraw the patient from the study if the participant revokes his/her consent to participate to the Registry.
After acceptance, demographic and clinical data are collected by the physician on a web-based Electronic Case Report Form (eCRF).
Patients enrolled for the study assume their own medical treatment according to the characteristics of their disease, as prescribed by the physician. All changes to the therapy are recorded

Figure 1 *- Geographic distribution of participating Italian Centers. The map shows how the 105 Centers partecipating to the Registry are geographically distributed. Cities with three or more participating Centers are reported as a big circle.*

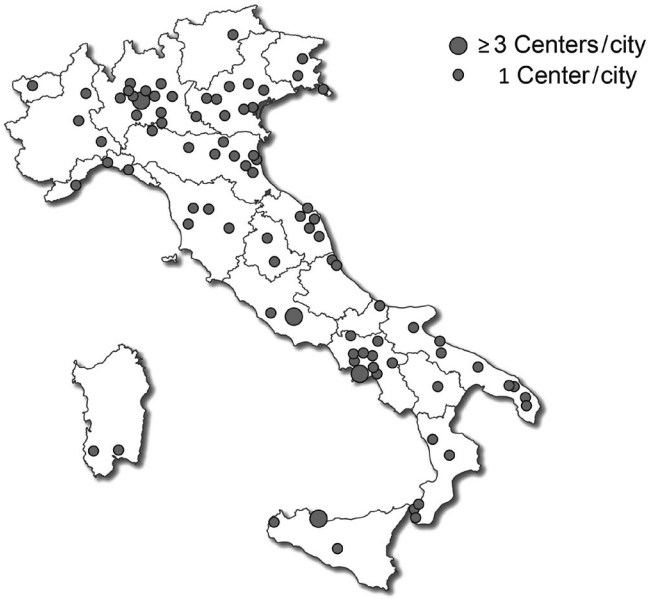
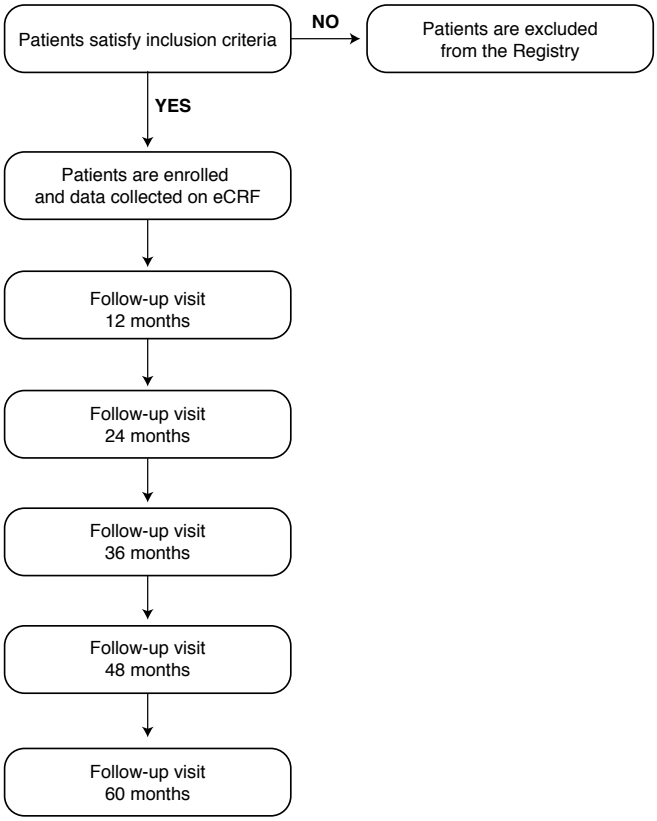


***Figure 2** - Flowchart. Schematic representation of the IRSA protocol.*



at the follow-up visit.
Subjects are followed until the end of the study.

Study population

Eligible patients are male or female ≥ 14 years of age, with a diagnosis of SA according to the GINA guidelines (http://ginasthma.org) on regular treatment with: i) high doses of inhaled corticosteroid (ICS) and long-acting β2-agonists (LABAs); or ii) med/high doses of ICS/LABA + leukotriene receptor antagonist (LTRA); or iii) med/high doses of ICS/LABA + theophylline; or iv) Oral steroids for at least 150 days/year + inhalation therapy. Patients with a clinical history of chronic obstructive pulmonary disease (COPD) or other respiratory diseases are excluded from the study.
All patients provided written informed consent prior to enrollment, and personal data used to identify patients were replaced with a study ID number prior to further data processing. The study was approved by the Ethics Committee Milan Area 3 of the Coordinating Center. However, for multi-center studies, multiple submissions to local institutional ethics committees are required, and satellite clinical sites can start enrollment only after having received the favorable opinion of the Ethical Committee. This is a no-profit study according to the Italian Law, D.M. 17.12.2004.

Data collection

Data of patients eligible for the study are collected on the eCRF and registered in the electronic database developed by CINECA (Bologna, Italy, www.cineca.it), a no-profit Consortium made up of 70 Italian universities, 8 Italian Research Institutions and the Italian Ministry of Education, operating in the management and development of web-based services. For each participating subject, the eCRF required the following information:

1. Demographic data (i.e. sex, high, weight, body mass-index);
2. History of smoking;
3. History of asthma and allergies;
4. Level of asthma control assessed by Asthma Control Test (ACT) or Asthma Control questionnaire (ACQ);
5. Number of exacerbations and hospitalizations in the 12 months preceding the visit;
6. Direct and indirect health costs;
7. Presence of co-morbidities (i.e. nasal polyposis, hypertension, diabetes, gastro-oesophageal reflux, obesity);
8. Laboratory data and functional tests (i.e. total IgE, leucocytes, neutrophils, lymphocytes, eosinophils, Forced Expiratory Volume in one second [FEV1], FEV1/Forced Vital Capacity [FEV1/FVC], FeNO);
9. Asthma therapy, including biologics;
10. Follow-up after 12 months from the first visit (ACT or ACQ score, exacerbations, laboratory data and functional tests, evaluation of effectiveness of medical treatment).

All the eCRFs are stored on-line in the central database for data processing, and analysis will be performed on aggregated data. The access to the eCRF is regulated by personal user credentials (user-id and password) linked to the Investigator responsible for data collection. Participating Centers are allowed to consult only their enrolled patients, and the access to information (registration/consultation of data) is encrypted by the Internet HTTPS protocol. Data are collected according to the European Regulation (EU) 2016/679 (General Data Protection Regulation - GDPR) and can be processed only with the informed consent of patient.

Ethical aspects and respect for confidentiality

The present study is carried out according to the Helsinki Declaration and National and International law on Observational Studies, in order to ensure the maximum protection for the subjects involved in the study. The proposed protocol is performed

according the Good Clinical Practice (GCP - ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996). The promoter of the study is committed to the protection of sensitive, clinical and personal data of the subjects involved in the study, as established by the Italian Legislative Decree n. 196/2003 (Data Protection Code) and by the latest GDPR 2016/679 (applicable in all the Member States of the European Union since 25 May 2018). Investigators are responsible for obtaining patient's informed consent after adequate information about the aims, methods, expected benefits and foreseeable risks of the study. Experimenters or agents should also inform participants that their participation or interruption will not cause modifications to the normal clinical practice.

Statistical analysis

This is an observational and descriptive study. The sample size was estimated assuming that the Severe Asthma disease has a prevalence of the 5% in the overall Italian patients affected by asthma. Based on the inclusion criteria, we estimate to enroll 4,800 patients affected by SA. In this context, we assume to be able to accurately estimate the prevalence of a specific characteristic (i.e. smoking). For example, supposing that the 30% of patients are smokers, the sample size of 4,800 subjects will produce a 95% confidence interval with an estimated error of 2.6%. Sample size was calculated using the PASS v.11 software (NCSS, LLC. Kaysville, Utah, USA).

Results will be represented as mean, median, standard deviations (SD), quartiles, and outliers, respectively for normally and non-normally distributed values. Data will be graphically presented as histograms, box-plots and whisker-plots. Tables of frequency distributions (numbers and percentages) will be prepared for categorical variables or with mean ± SD for continuous variables.

Comparisons of specific subgroups of data will be done with the Chi-square test or the exact Fisher test (for categorical variables), or with the two tailed t-test or the non-parametric test "Rank-sum" of Wilcoxon (for continuous variables). Differences among comparisons groups will be determined by the analysis of variance (ANOVA) for repeated measurements or, in the case of non-homogenous distribution, by the non-parametric Kruskal-Wallis test. Statistical analysis will be performed using the Statistical Analysis Software v 9.4 (SAS) (SAS Institute Cary, NC, USA).

Conclusions

Severe asthma is a heterogeneous disease in adults as well as in adolescents, with different clinical and inflammatory phenotypes. SA treatment is particularly challenging due to the high morbidity and disease-related costs. Biologic therapies represent a significant opportunity for customized treatment to patients who do not respond to traditional asthma therapy, but a more accurate selection of patients with SA is needed. Despite this, the routinely use of reliable and non-invasive biomarkers in patients whose asthma is refractory to standard therapy is still missing. For all these reasons, there is an urgent need to characterize patients affected by severe uncontrolled asthma in *real life*. Several registries collecting cases of SA have been recently created in different countries, each of them with their strengths and weaknesses.

For the first time in Italy, the IRSA project will give the opportunity to understand the epidemiological, clinical and therapeutic aspects related to the pathology, filling the gap between adolescents and adults, identifying risks factors, analyzing multiple clinical phenotypes, and monitoring treatments safety and efficacy.

We believe that collecting data across a large range of ages will provide new information, useful to improve asthma health care and the management of affected patients, according to the best clinical practice.

Conflict of interest

The authors declare that they have no conflict of interest.

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Effect of industrial processing on the IgE reactivity of three commonly consumed Moroccan fish species in Fez region

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Key words

allergenicity; fish products; human IgE; rabbit IgG; industrial processing

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Summary

Objectives. *The aim of this work was to study the effect of industrial processing on the allergenicity of three commonly consumed Moroccan fish species in Fez region (sardine, common pandora, and shrimp).* ***Methods.*** *This work was conducted by a sera-bank obtained from 1248 patients recruited from Fez Hospitals. Their sera were analyzed for specific IgE binding to raw fish extracts. Among them, 60 patients with higher specific IgE levels were selected, and used to estimate the binding variation of IgE to these products under several processing (frying, cooking, canning, marinade, and fermentation) using ELISA analysis.* ***Results.*** *ELISA results demonstrated that all the studied processing cause a reduction in the immunoreactivity of human IgE to fish products, with a high action with marinade and fermentation compared to other processing. This alteration was also observed with rabbit IgG in all processed products, showing that the maximum reduction was marked in fermented sardine with 64.5%, in cooked common pandora with 58%, and in fermented shrimp with 69.2%.* ***Conclusion.*** *In conclusion, our study has shown that the allergenicity of the three studied fish could be reduced by different industrial processes with different degrees.*

Introduction

Fish and shellfish are important foods in the world, including Morocco. Thus, fish and shellfish play an important role in human nutrition and health. They are a valuable source of proteins, physiologically active substances such as eicosapentaenoic acid and docosahexaenoic acid, and minerals such as calcium. However, fish and shellfish mediated by immunoglobulin E (IgE) are becoming a serious problem worldwide, especially in coastal regions (1-4). In Morocco, previous works by our laboratory showed that 9.5% of 2802 children, aged between 12 and 18 years, reported an allergy to fish and shellfish (5).

Among the various allergens characterized in fish and shellfish, parvalbumin (12-13 kDa), a calcium-binding muscle protein, has been identified as the major fish allergen (6-8), while tropomyosin (35-38 KDa), a myofibrillar protein, has been recognized as the major shellfish allergen (9-11). Several researches have demonstrated that food processing techniques may induce changes in protein conformation, which may thus affect allergenicity if the allergen epitopes are modified by the process (12-15). Fish and shellfish are subjected to a wide variety of processes, such as thermal (cooking, frying), freezing, canning, salting, drying and fermentation, any of which might reduce or increase their allergenicity.

In the present study, fresh and processed fish (fried, cooked, canned, marinated, and fermented products) have been studied. The aim of this work was to study the effect of industrial processing on the allergenicity of three commonly consumed Moroccan fish species in Fez region (sardine, common pandora, and shrimp). The effects of processing on the allergenicity of these

products have been analyzed by measuring IgE binding in sera from patients with high raw fish-specific IgE levels, using ELISA.

Materials and methods

Fish samples

Samples of three commonly consumed fish species (sardine, common pandora, and shrimp), fresh and processed, were purchased from various local stores in the city of Fez. The whole fish was rinsed briefly with distilled water. The raw, fried, cooked, canned, marinated and fermented muscle extracts of the 3 fishes were prepared, and each extract was tested by IgE- and IgG-ELISA with patient's sera.

Preparation of fish extracts

Preparation of sardine and common pandora extracts

The raw extract of each fish was prepared as previously described (16,17). Briefly, fish muscles were defatted with chloroform (20%) to remove lipids. After filtration, the powder was dried overnight at room temperature. 10 g of samples were subsequently extracted in 100 ml of phosphate buffer solution (PBS) (pH 7.4) for 12 h at 4 °C. The mixture was then centrifuged at 3000 rpm for 15 min, and the supernatant was filtered and stored at -20 °C until use. Quality of extracted proteins was evaluated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Each fish species was fried with vegetable oil for 5 min and subsequently placed on filter paper to remove oil. The cooked fish extract was prepared by boiling fish in water for 10 min. Marinade fish was prepared with olive oil, lemon juice, salt, pepper and garlic for 3 days at 4° C. Canned sardine was purchased from national markets. The last group of fermented fish occurred by natural fish flora; the fishes were mixed with salt and then fermented for two weeks. All these fish samples were extracted as described above with raw extracts.

Preparation of shrimp extracts

The raw shrimp extracts were prepared as previously described (18). Briefly, shrimp muscle (5 g) was incubated in 50 ml of extraction buffer (1 mol/L KCl and 0.5 mmol/L β-mercaptoethanol, pH 7.0) for 16 h at room temperature. Supernatants were collected by centrifugation (12,000 xg, 15 min, 4 °C) and stored at -20 °C until use. Quality of extracted proteins was evaluated by SDS-PAGE.

Concerning industrial processing of shrimp, samples were subjected to different treatments including frying with vegetable oil (for 3 min), cooking/boiling in water (for 5 min), marinade with olive oil, lemon juice, salt, pepper and garlic (for a day at 4 °C), and fermentation "shrimp paste", which it is primarily made from finely crushed shrimp mixed with salt, and then fermented for several weeks. Shrimp paste was purchased from national markets. The samples (5 g) were extracted, immediately after processing, in 50 ml of extraction buffer and centrifuged as above (raw extracts). All the extracts were preserved at -20 °C for further experiments.

SDS-PAGE analysis

Protein samples (100 µl per well) were mixed with loading buffer (10% SDS, 10% glycerol, 10% β-mercaptoethanol, and 2.5% bromphenol blue), heated at 100 °C (5 min), electrophoresed in 15% analytical SDS-polyacrylamide gels, and resolved by SDS-PAGE. Proteins were stained using Coomassie Brilliant Blue R-250.

Human sera

The study was conducted on a sera-bank, with data obtained from 1248 patients recruited in Fez city from the University Hospital Centre and from Fez laboratory. It should be noted that these patients chosen at random were coming from different medical tests. Only patients under anti-allergic treatments were excluded. A questionnaire was provided to all patients concerning food allergy characteristics and fish allergies in particular. Patients were asked to provide information on the presence of allergic symptoms and about their family allergy history. This work was conducted from May 2014 to June 2015, and was approved by the Ethic committee of the University Hospital Center of Fez. After formal consent of the patients, a blood sample of 3 ml was collected in a dry tube. After centrifugation at 3000 rpm during 5 min, sera were separated and stored at -20 °C until use.

ELISA analysis

Among 1248 patients, 1008 were analyzed for specific IgE to sardine. From the 1008 patients, 500 have been analyzed for specific IgE to common pandora. In parallel, 260 patients (from 1008) and other 240 patients were analyzed for specific IgE to shrimp. Among each group, 20 patients with high specific IgE levels (> 80 IU/ml) to raw fish extracts were selected, and used to estimate the binding variation of IgE to processed fish extracts using enzyme-linked immunosorbent assay (ELISA).

Specific IgE binding to raw and processed fish was assayed by Indirect ELISA as previously described (16-20). Briefly, 100 µl of fish protein extracts (0.5 mg/ml) was deposited per well in 96 well microplates and incubated for 60 min at 37 °C. Then, 200 µl of 0.5% bovine serum albumin (BSA) was added to every well for an hour at 37 °C. After removal of BSA, human sera were added (100 µl/well) before incubation with goat anti human IgE peroxidase conjugate for 60 min at 37 °C. Bending of anti-IgE was revealed by adding 100 µl of 0.05% OPD (orthophenylene-

diamine). The reaction was stopped by adding HCl 3M. Then the developed color was measured by absorbance at 490 nm.

Preparation of polyclonal antibodies against fish and shellfish extracts

To study the immunoreactivity of IgG antibodies to sardine, common pandora and shrimp extracts, rabbit IgGs were prepared against native fish and shellfish extracts (sardine, common pandora and shrimp). These antibodies were obtained after repetitive immunization of rabbits against the native fish and shellfish extracts, using Freund adjutants as described before (16-18,20). After one month, the animals were sacrificed, and blood samples were collected in dry tubes and sera were separated. Then, sodium azide 0.1% was added to the sera and frozen at -20 °C until use.

Statistical analysis

Descriptive statistics were presented as numbers with percentages or as average values. Statistical analysis was based on the student's t-test taking $p < 0.05$ as the limit of the significant value.

Results

Sample description

In our study, we observed that the self-reported food adverse reactions in 1248 patients were 10.3%. Regarding the results of allergy to fish and shellfish, we noticed a strong sensitivity of this population to the fish products (7.5%). From the 7.5% (n = 93) who reported fish/shellfish allergy, we noted that the prevalence according to sex was higher in female (75%) than in male (25%), and 35.5% of them have an allergic history in their family. From this sensitive population, the most frequent clinical signs were cutaneous reactions (75.3%), followed by gastrointestinal reactions (25.8%), and respiratory symptoms (9.7%). Regarding the allergic self-reported reactions in relation to the different food processing in our study population, we observed that fried fish/shellfish (91%) was the most frequently reported, followed by cooked (67%) and canned fish/shellfish (39%).

Specific IgE of patients

For sardine, sera of 1008 patients have been tested for specific IgE binding to sardine, and the results showed that 27.3% (275/1008) of patients exhibited levels more than 80 IU/ml. From these sensitive patients, we have selected 20 persons (10 children: 5 female, 5 male; 10 adults: 9 female, 1 male) demonstrating high specific IgE to raw sardine with an average value of 199.79 IU/ml, varying between 103.17 to 330.67 IU/ml.
For common pandora, sera of 500 patients have been analyzed for specific IgE binding to common pandora. The results indicated that 25.6% of patients (128/500) presented IgE values higher than 80 IU/ml, of which 11% (55/500) had cross-reactivity with sardine. From them, we have selected 20 persons (5 children: 2 female, 3 male; 15 adults: 12 female, 3 male) displaying high IgE reactivity to raw common pandora with an average value of 121.54 IU/ml, varying between 80.25 and 289.42 IU/ml.
For shrimp, the dosage of specific IgE to shrimp in 500 patients has shown that 20% of patients (100/500) presented IgE values higher than 80 IU/ml, of which 3% (8/260) had cross-reactions to sardine-specific IgE. Among this group, we have selected 20 persons (3 children: 2 female, 1 male; 17 adults: 15 female, 2 male) demonstrating high specific IgE to raw shrimp with an average value of 173.79 IU/ml, varying between 136.5 and 220.67 IU/ml.
Distribution of specific IgE levels to raw extracts of the three fish species among the studied population (20 per group) is summarized in **table I**.

Table I *- Distribution of specific IgE Levels to raw extracts of 3 fish species among the studied population.*

Demographic variable	Sardine-specific IgE levels; n = 20		Common pandora-specific IgE levels; n = 20		Shrimp-specific IgE levels; n = 20	
	80-150 IU/ml	> 150 IU/ml	80-150 IU/ml	> 150 IU/ml	80-150 IU/ml	> 150 IU/ml
All	3	17	17	3	2	18
gender						
female	2	12	12	2	1	16
male	1	5	5	1	1	2
age						
children (< 20 years)	1	9	4	1	1	2
adults (> 20 years)	2	8	13	2	1	16

Effect of industrial processing on fish proteins using SDS-PAGE analysis

Protein profiles of raw and processed fish protein extracts were analyzed by SDS-PAGE. As shown in **figure 1**, SDS-PAGE profile of sardine and common pandora proteins showed a dark band, corresponding to parvalbumin (PV) at approximately 12 KDa, which is considered to be the major allergen in fish. These were equally prominent in fried and in cooked extracts. The migration of canned extract indicated loss of definable PV bands with smear profile, suggesting probably a degradation of proteins (A, lane 5), while the PV bands disappeared completely in all fermented extracts (A, B, lane 6) as well as in all marinated extracts (A, lane 7; B, lane 5).

Additional protein bands were seen in the raw sardine at 34, 36 and 40 KDa (A, lane 2). Under processing, the 36-KDa band was observed only in fried and cooked sardine extracts, while the 34 and 40 KDa bands disappeared and faded away in all processed sardine extracts. The raw common pandora showed several additional bands between 14 and 45 KDa. These bands were also seen in fried extract, but some new minor bands appeared at about 34 KDa in fried pandora extract. The cooked extract displayed approximately at 34-36 KDa a much more prominent band in comparison to the raw and fried extracts. Besides, some new minor bands ranging from 20 to 34 KDa appeared in cooked extracts. Contrarily, no protein band appeared in marinated and fermented common pandora.

Concerning SDS-PAGE profile of shrimp proteins, tropomyosin (TP), considered the shellfish major allergen, appeared at 36-38 kDa. This band was also observed in fried and cooked shrimp extracts, while no TP bands were visible in marinated and fermented extracts. Additional minor protein bands were observed in the raw shrimp at approximately 55 KDa and at > 66 KDa. Under frying and cooking processing, the > 66 KDa band was seen only in the fried extract, while the 55 KDa band faded away in the fried and in the cooked extracts. These bands disappeared completely when shrimp was marinated or fermented.

Effect of industrial processing on the detection of fish proteins extracts by rabbit IgG using ELISA

The variation in immunoreactivity of fish/shellfish protein extracts after processing was assessed by rabbit IgG (anti-raw extracts) (**figure 2**) and by human IgE. The results showed that the IgG binding to sardine proteins was decreased in all processed extracts (**figure 2**). It was decreased by 19.8% in fried sardine, 27.2% in canned sardine, 29% in cooked sardine, 53.4% in marinated and 64.5% in fermented ones, compared with raw sardine. Concerning the ELISA results of the common pandora extracts under different treatments, results showed that there was a significant decrease in the binding of anti-IgG in all processed extracts (**figure 2**). This binding was reduced by 39.7%, 58%, 41.8% and 49.8%, respectively, in the fried, cooked, fermented and marinated common pandora. As regards shrimp extracts (**figure 2**), we noted a significant reduction by 69.2% in IgG binding in the fermented shrimp, as well as in the marinated shrimp, which was decreased by 37.2%. No significant

Figure 1 *- SDS-PAGE profiles of raw and processed fish protein extracts. A, sardine protein extracts (Sardina pilchardus); B, common pandora protein extracts (Pagellus erythrinus); C, shrimp protein extracts (Penaeus spp). Abbreviations: Mr, markers of the molecular weights; PV, parvalbumin; TP, tropomyosin.*

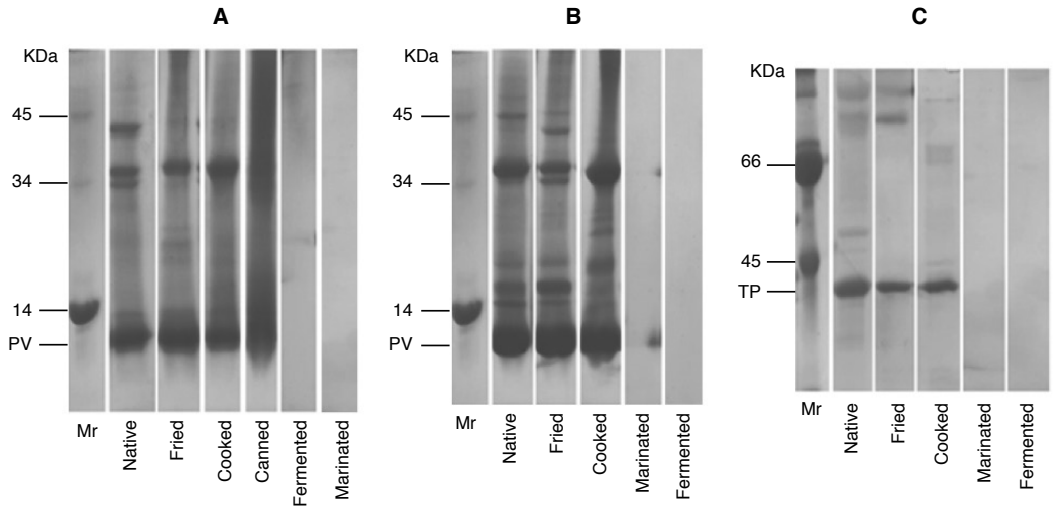
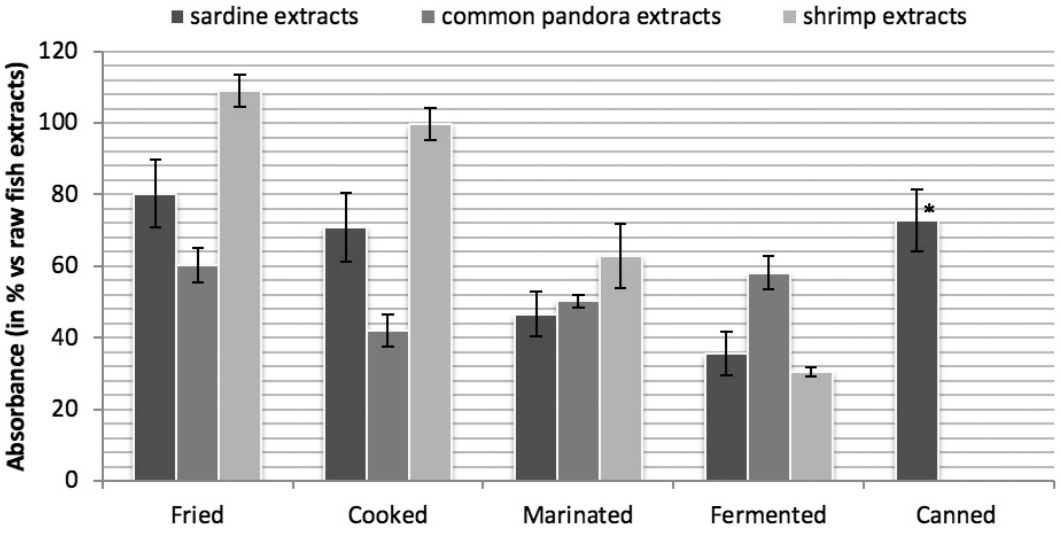


Figure 2 *- Effect of industrial processing on the recognition of sardine, common pandora and shrimp extracts by rabbit IgG.*



[1]Canned treatment was done just for sardine species.

change was observed in cooked extracts. In contrast, the fried treatment showed an increase in the IgG binding.

In general, all the processed sardine and common pandora extracts showed a decrease in the IgG binding. In sardine extracts, processing gradually decreased the capacity of the IgG binding in the following order: raw > fried > canned > cooked > marinated > fermented, whereas in common pandora extracts, it was: raw > fried > fermented > marinated > cooked. However, in processed shrimp, it was in the order: raw ~ cooked > marinated > fermented.

Effect of industrial processing on human IgE binding to fish proteins extracts using ELISA

ELISA was performed using selected human sera with high IgE binding levels to raw sardine (n = 20), raw common pan-

Figure 3 *- Effect of industrial processing on the recognition of sardine extracts by human IgE.*

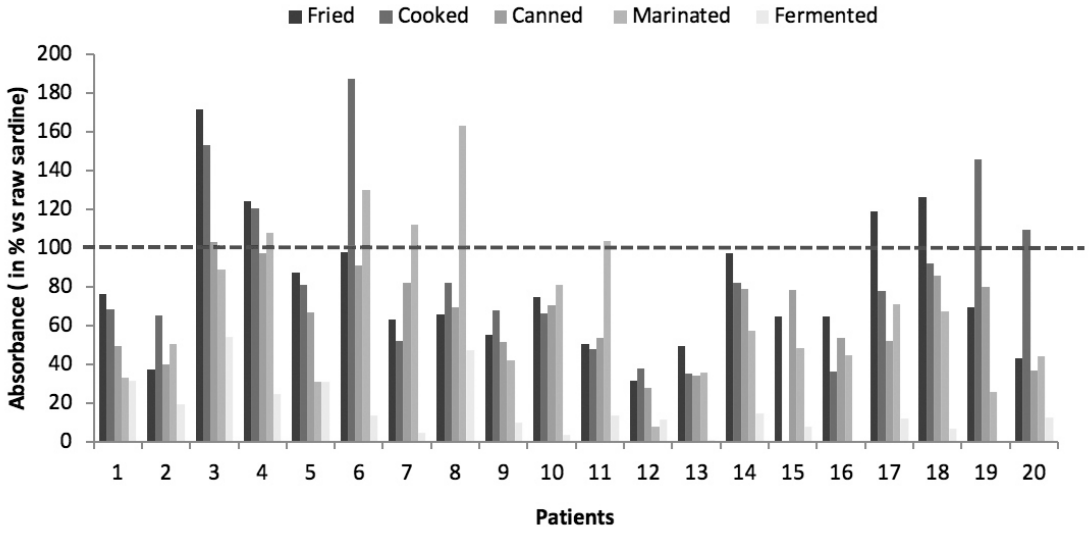


Figure 4 *- Effect of industrial processing on the recognition of common pandora extracts by human IgE.*

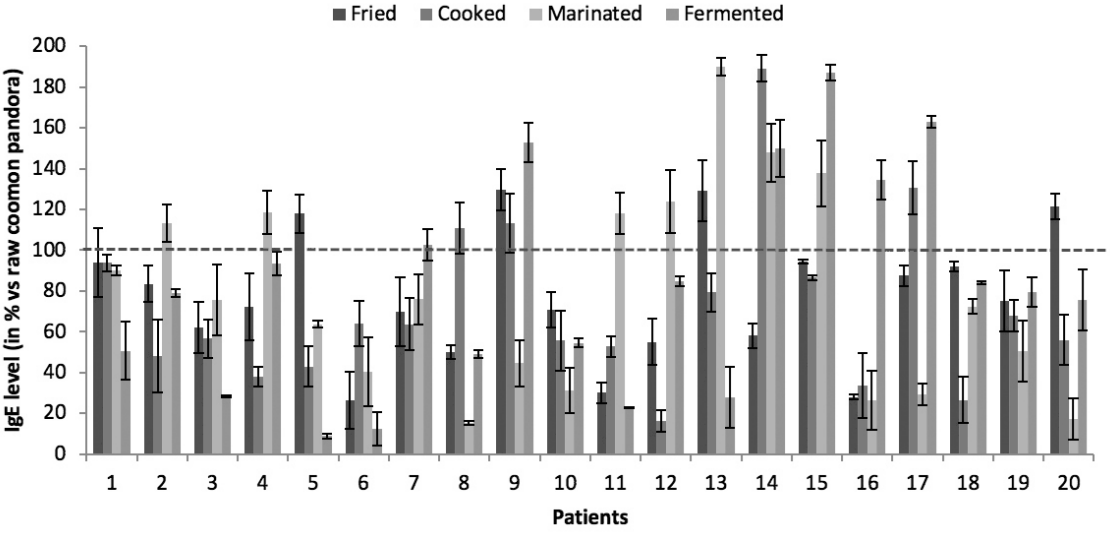
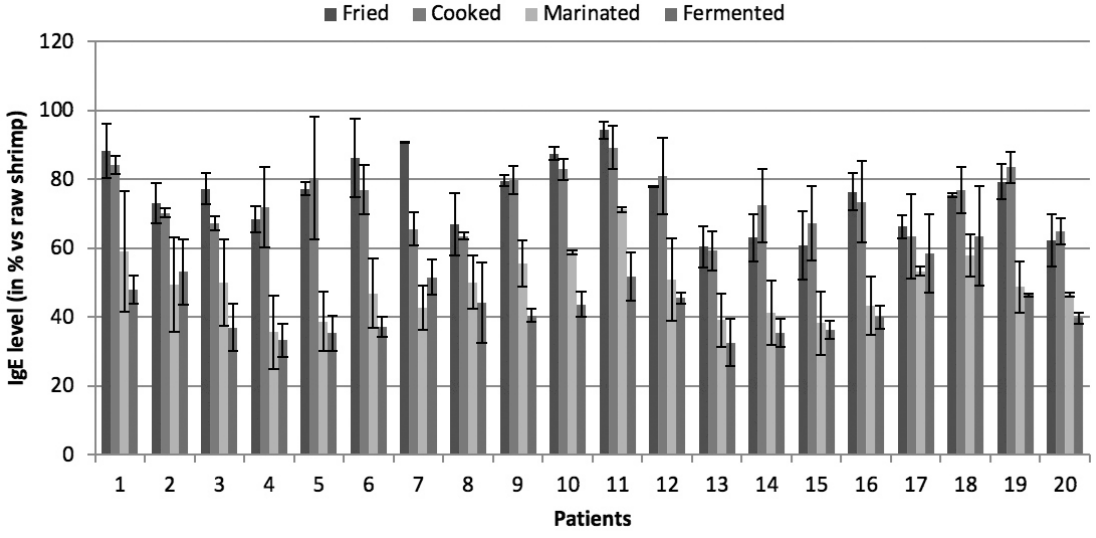


Figure 5 *- Effect of industrial processing on the recognition of shrimp extracts by human IgE.*



dora (n = 20), and raw shrimp (n = 20). These sera were used to estimate the binding variation of IgE to fish species under different processing (**figures 3**, **4** and **5**). The recognition of raw fish species by human IgE was used as a control.

Figure 3 represents the distribution of specific IgE-binding levels to processed sardine proteins within a population of 20 patients. A marked decrease in the IgE binding was seen in the majority of patients. In this case, fermented sardine showed reduced IgE binding in all tested patients, with an average diminution of 84%. Frying, cooking and canning products demonstrated decreased IgE binding in most sera (16/20, 15/20 and 19/20, respectively) with an average diminution of 35.6%, 40.4% and 36.8%, respectively. In canned case, we observed that 8 patients exhibited more than 50% of IgE levels reduction. Similarly, marinade products showed decreased IgE binding in 15/20 patients, with an average diminution of 51.3%. Increased IgE binding was seen in 3 products for two sera (no. 3, 4), in 2 products for one serum

(no. 6) and in 1 product for seven sera (no. 7, 8, 11, 17, 18, 19, 20).

In processed common pandora (**figure 4**), frying and cooking products both showed decreased IgE binding in 16/20 patients, with an average diminution of 34.4% and 44.9%, respectively, while marinade and fermented products showed a reduction in the binding of IgE in 13/20 and 14/20 patients, respectively, with an average diminution of 51.4% and 46.4%, respectively. Increased IgE binding was seen in 3 products for two sera (no. 9, 14), in 2 products for three sera (no. 13, 15, 17), and in 1 product for nine sera (no. 2, 4, 5, 7, 8, 11, 12, 16, 20). In processed shrimp, **figure 5** showed a decrease in the IgE binding to fried, cooked, marinated and fermented shrimp in all tested patients, with an average diminution of 24.4%, 26.3%, 51.1% and 56.3%, respectively.

Discussion

The objective of this work was to study the effect of industrial processing on the allergenicity of three commonly consumed Moroccan fish species in Fez region (sardine, common pandora, shrimp). From human bank sera, 60 patients with high specific IgE levels to raw fish/shellfish extracts were selected, and used to estimate the binding variation of IgE to these products under several processing (frying, cooking, canning, marinade, and fermentation) using ELISA assay.

On SDS-PAGE profile of sardine and common pandora proteins, the parvalbumin was detected in fried and cooked extracts with almost the same band intensity as that in the raw extract, suggesting that the sardine and common pandora parvalbumin is still stable after frying and cooking. However, fermentation and marinade processing caused disappearance of parvalbumin band as well as all the additional bands. This suggests that parvalbumin is unstable on these processing methods, probably including degradation of fish proteins in small parts which were not revealed by SDS-PAGE. Similar conclusion by Sletten et al. (21) showed that parvalbumin was less stable in chemically processed fish types, fermented trout and some salted fish products. In canned sardine, loss of definable bands was observed on SDS-PAGE profile protein, compared to the raw extract. Similarly, alteration of clearly definable protein bands in canned tuna and salmon has been previously reported (21,22). Concerning SDS-PAGE profile of shrimp proteins, the tropomyosin was seen in fried and cooked extracts, suggesting that shrimp tropomyosin is stable under those heating conditions (frying for 3 min and boiling for 5 min). Whereas, this band protein disappeared when shrimp was marinated or fermented.

In a second part of the study, we have analyzed by ELISA the IgE immunoreactivity to processed sardine, common pandora and shrimp extracts using sera with high specific IgE levels to raw extracts. The results revealed that the fried and the cooked extracts showed a decrease in the IgE binding in the majority of patients, with an average reduction ranging from 25% to 45%. Similarly, boiling and frying have been reported to reduce IgE binding properties of the fish allergens depending on fish species (23). On the other hand, we haven't observed any modification in fish/shellfish allergens bands by SDS-PAGE, so the low reduction observed by specific IgE analysis can be explained by modification of conformational IgE binding epitopes induced by heating conditions, since we observed approximately the same level of IgE reduction in boiling and frying processing. Previous works by our laboratory found that the IgE binding activity to isolate sardine parvalbumin, common pandora parvalbumin and shrimp tropomyosin can be reduced by heating, which was confirmed by dot blot assay showing that the intensity of spots became weak in all tested patients (16-18). This allowed us to consider that fish/shellfish allergen undergoes conformational changes depending on the heating load, resulting in a decrease in the binding ability to specific IgE. This suggests that the allergen epitopes recognized by human IgE are partially conformational. In contrast, increased IgE binding to processed extracts was seen in some patients, suggesting that some epitopes seem to be sequential. On these data, we concluded that boiling and frying can reduce the IgE binding to fish products, but they are not a solution for patients allergic to fish due to the low reduction in the IgE binding caused by these processes. This was confirmed by our questionnaire, showing that more than 60% of patients reporting sensitivity to fish declared allergy to fried and cooked fish products.

In canned sardine, our results demonstrated that canning products showed a high reduction in the human IgE binding in 95% of patients, with an average diminution of 36.8%, of which 8 patients exhibited more than 50% of IgE levels reduction. Similar results were described by different authors, who reported that canned tuna and salmon are less allergenic than raw and cooked fish, showing that the allergenicity of fish parvalbumin can be reduced during canning process. This decrease was demonstrated by ELISA-inhibition and oral challenges (21,22,24). A recent descriptive study from Australia found that more than 21% of children allergic to salmon or tuna were able to tolerate the fish in canned form, indicating a reduction in skin prick test (SPT) size in most patients (25). Interestingly, Bernhisel-Broadbent et al. (22) showed that decreased fish allergenicity in canned tuna has important clinical implications, suggesting that canned products provide a nutritious, low fat, high protein food that can be recommended to many patients with fish allergies. Regarding our results and previous works, we could suggest that people allergic to fish could tolerate canned products. This was confirmed by our questionnaire, which revealed that only 39% of persons reporting fish allergy declared allergy to canned products compared to fried (91%) and cooked (67%) products.

Regarding marinade processing, we noted that marinade fish products (sardine, common pandora and shrimp) showed a reduction in the binding of IgE in the most of patients, with an average diminution exceeding 50%. By fermentation processing, results demonstrated that all tested patients showed a loss in their IgE reactivity to fermented sardine and shrimp products, with an average reduction of 84% and 56.3%, respectively. In fermented common pandora, we noted a less effect than that observed with sardine and shrimp, with an average diminution of 46.4% marked in the most of patients. This decrease in the fish/shellfish allergenicity after fermentation processing can be explained by the action of microbial flora, causing proteolysis of fish/shellfish allergens and loss of IgE binding epitopes. During marinade fish processing, microorganisms may cause degradation of fish/shellfish proteins and therefore denaturation of the IgE binding epitopes, leading to a reduction in allergenicity. This was confirmed by SDS-PAGE, showing an absence of bands in marinated and in fermented extracts. These suggestions were confirmed by our data obtained before, showing a reduction of the allergenicity of sardine parvalbumin, common pandora parvalbumin and shrimp tropomyosin after enzymatic digestion, demonstrated by ELISA and dot blot analysis (16-18). Interestingly, previous studies showed that curing and fermentation processing resulted in a loss of IgE binding, suggesting that these processing are enzymatic processes which may induce changes in parvalbumin oligomers, either by altering the conformation or by direct cleavage of the IgE binding epitopes (21,26). Furthermore, other works studying the effect of fermentation on the allergenicity of food proteins found that fermentation may destroy some antigenic epitopes, resulting in decreased allergenicity (27-30). This high action by marinade and fermentation processing was also observed with rabbit IgG, showing a high significant decrease in the IgG binding compared to frying and cooking. From these results, we concluded that marinade and fermentation processing can constitute an alternative for fish/shellfish allergic patients to consume fish/shellfish without allergic reactions, since the observed reduction was greater than cooking or frying.

Conclusions

In conclusion, our study has shown that the allergenicity of three commonly consumed Moroccan fish species can be reduced by different industrial processes (frying, cooking, canning, marinade and fermentation) with different degrees. Among these processing, marinade and fermentation seem to be more effective in the reduction of the IgE reactivity, compared to other processing.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Drug-induced anaphylaxis: seven-year single-center survey

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Keywords
allergological workup; anaphylaxis; antibiotics; drug hypersensitivity; nonsteroidal anti-inflammatory drugs

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Summary
Background and objective. *Drug-induced anaphylaxis (DIA) is the most common cause of fatal anaphylaxis. We aimed to characterize patients with DIA and their allergological workup.* ***Methods***. *Systematic review of patients with history of DIA referred to our center over 7 years.* ***Results***. *125 patients were included (10% pediatric age), the median age of first episode being 36 years (ranging from 1 to 74 years). The main culprits were nonsteroidal anti-inflammatory drugs (NSAIDs) (43%), antibiotics (42%) and anesthetic agents (6%). In 24% of cases the reactions occurred in hospital setting and 14% were perioperative. The etiology was confirmed in 75% of cases through allergological workup.* ***Conclusions***. *NSAIDs and antibiotics were responsible for most of DIA. The heterogeneity of mechanisms, the severity of the reactions and the lack of standardized in vivo and/or in vitro tests for some drugs do not allow to confirm the diagnosis in all cases. Patients with DIA should be evaluated in specialized centers to perform accurate diagnosis, to prevent recurrence and to find safe alternatives.*

Introduction

Anaphylaxis is a rapid-onset, severe and potentially life-threatening systemic hypersensitivity reaction. It is usually characterized by airway, breathing, or circulatory manifestations and usually, although not always, associated with skin and mucosal symptoms (1). Anaphylaxis results from immunological (immunoglobulin E [IgE] or non-IgE mediated) or non-immunological mechanisms to certain antigens, with release of vasoactive mediators from tissue mast cells and peripheral basophils. Regarding drug anaphylaxis, the reactions are mostly mediated by antigen-specific IgE responses, but other mechanisms have been clarified (2). Most drugs are relatively too small to elicit an immune response, and are supposed to act as haptens or prohaptens in order to be immunogenic (IgE-dependent mechanism). IgE-independent drug anaphylaxis might result from direct mast cell degranulation, complement activation, kallikrein activation, histamine release or due to contaminants in drug formulation (3).

Drug-induced anaphylaxis (DIA) is the most common cause of fatal anaphylaxis (4,5). According to Jerschow et al., 58.8% of fatal anaphylaxis were drug-induced. Nevertheless, in 75% of the fatal cases the culprit drug was not specified (5).

Anaphylaxis related to nonsteroidal anti-inflammatory drugs (NSAIDs) is typically drug-specific or class-specific, as well as with beta-lactams antibiotics (BL). NSAIDs have been considered as the most common pharmacological cause of anaphylaxis (6). Some of these reactions occur with the whole class or appear to be drug-specific (3).

Although skin testing and drug provocation tests (DPT) can confirm the diagnosis, in severe cases the diagnosis is mostly based on clinical history. Furthermore, the main difficulty in confirming the diagnosis of DIA results from the lack of standardized tests to most drugs, and often the patients refuse to

undergo the challenge tests with the culprit drug, considering the severity of the reactions reported.

It is difficult to quantify the rate and mortality of DIA, due to the heterogeneity of methodology, different sample populations and definitions of anaphylaxis. Furthermore, the notification systems are not uniform (7). The estimated incidence of anaphylaxis in Western countries ranges from 8 to 50/100,000 person-years, with a lifetime prevalence of 0.05 to 2% (8). According to older studies, penicillin and anesthetic agents given during the perioperative period were the commonest causes of IgE-mediated allergic anaphylaxis, while NSAIDs and radiocontrast media were the most common causes of non-immunological anaphylaxis (6). Drugs are considered the main trigger of adult-age anaphylaxis, with the highest rate observed in the 55 to 84 year age group (3.8/100,000 individuals), and were the main cause of fatal anaphylaxis in the United Kingdom, New Zealand and Australia (7).

We aimed to characterize patients with DIA and their drug allergy workup, contributing to a better understanding about the main elicitors and the accuracy of a proper allergological investigation.

Materials and methods

A systematic evaluation of all patients with clinical history compatible with DIA reported to our drug allergy center over seven years (from January 2010 to December 2016) was performed. For each patient, the allergist filled in a questionnaire with all clinical information related with the episode and the investigation performed. The patients were investigated according to ENDA/EAACI (European Network of Drug Allergy/European Academy of Allergy and Clinical Immunology) recommendations, through skin testing (9) and specific IgE to drugs when they are standardized, and/or DPT when indicated (10,11). All patients signed an informed consent. Serum-specific IgE antibodies (ImmunoCAP®, Thermo Fisher Scientific) and skin testing were performed at least after a 4 weeks interval after the clinical reaction. For specific IgE a cut-off value of ≥ 0.35 kU/L was considered positive (12). Regarding drugs with standardized skin test concentrations, skin prick tests (SPT) were the first step of the in vivo investigation, and only if negative, intradermal tests (IDT) were carried out. For the BL workup we have used solutions of benzylpenicilloyl octa-L-lysine (PPL), sodium benzylpenilloate - minor determinant (MD), penicillin G, amoxicillin and clavulanic acid; other BL were tested if they were the culprit drug. PPL and MD were always performed with DAP® Penicillin extracts (Diater, Madrid, Spain). In the last two years, we also performed amoxicillin and clavulanic acid with DAP®-3 Amoxicillin and DAP® Clavulanic extracts (Diater, Madrid, Spain). IDT were carried out beginning with 10-100 times more diluted solutions, which were gradually increased until the appearance of a positive skin response, or until reaching the maximum concentration recommended for that drug (9). Histamine (10 mg/mL) was used as a positive control for SPT, and 0.9% saline solution as a negative control. In SPT, a mean wheal larger than 3 mm, accompanied by erythema, with a negative response to negative control, was considered positive. For IDT, 0.02 to 0.05 mL of solution is injected into the dermis to produce a small wheal, that is outlined; IDT was considered positive when the mean diameter of initial wheal increased more than 3 mm.

In case of negative results with in vitro and skin tests, and if needed to confirm diagnostic, the patients underwent DPT with the culprit drug. All in vivo tests were performed by allergists with experience in recognition and management of acute reactions. Epinephrine and other appropriate medication and resuscitation equipment were always available during the procedures.

Results

We report data from 125 patients, who were referred to our drug allergy center with a diagnosis of DIA performed by an allergist. We included patients from all ages, 10% aged under 18 years and 6% older than 65 years. Detailed demographic and clinical characterization is presented in **table I**.

The mean age at the first anaphylactic episode was 36.4 years, ranging from 1 to 74 years. Regarding the clinical manifestations, there was a predominance of mucocutaneous symptoms (96%), followed by respiratory (80%) and cardiovascular (45%) involvement, with loss of consciousness in 20% of cases and less frequently with gastrointestinal complaints (21%). In 68% of cases the reaction occurred within the first 30 minutes after drug administration.

Table I *- Demographic and clinical characterization.*

Baseline characteristics	
n (sex)	125 (66% female)
mean age at the first appointment (years) (± SD); (range)	41.1 (± 16.7); (1.5 - 75)
median age at the first anaphylactic episode (years); (range)	36.4 (1 - 74)
under 18 years	10%
atopic[1]	69%
asthma	25%
patients with recurrent episodes DIA	19%

[1]Atopy was defined as positive skin prick test to at least one common aeroallergen.

Abbreviations: DIA, drug-induced anaphylaxis; SD, standard deviation.

Concerning the culprit drugs, the main causes were NSAIDs (54 patients) and antibiotics (52 patients). Other drug agents found were proton pump inhibitors (6 patients), neuromuscular blocking drugs (5 patients), carboplatin (3 patients), corticosteroids (2 patients), local anesthetics (2 patients), ranitidine, midazolam and patent blue (1 patient each). **Table II** describes all the drugs involved, according to age group. Two patients reacted subsequently with two drug classes (antibiotics and NSAIDs): one of them with penicillin and metamizole, and the other with minocycline and metamizole.

The circumstance in which the reaction occurred was documented. We found that 24% of reactions occurred in hospital setting, and 14% of patients had perioperative anaphylaxis. Despite the severity of the reactions, only 40% of patients were appropriately treated with epinephrine. However, 22% could not remember exactly which medications were administered.

We included 18 patients with perioperative anaphylaxis. The agents involved in these reactions were mainly antibiotics (eight patients) and neuromuscular blocking drugs (five patients), followed by NSAIDs (2 cases; metamizole and ketorolac), midazolam, local anesthetic (bupivacaine) and patent blue dye (1 patient each). Among antibiotics we had seven cases with cefazolin and one with ciprofloxacin. Among neuromuscular blocking drugs we had three cases with atracurium, one with rocuronium and one with succinylcholine.

Furthermore, 19% of patients had recurrent episodes of DIA, before the etiologic diagnosis was made. Most of the recurrent cases were due to NSAIDs, what supports the diagnosis of DIA.

Etiologic diagnosis of DIA was confirmed in 94 patients (75%), through skin tests in 72 patients (**table III**) - we highlight that two patients had a severe systemic reaction during IDT that resolved with intramuscular epinephrine - and the remaining 22 patients by in vitro tests (2 patients) or DPT (20 patients). Considering the severity of reactions and the lack of standardized tests for some drugs, the remaining patients whose DIA was based on clinical history were successfully challenged with alternative drugs. In most of these cases, the culprit drugs were NSAIDs, COX-1 preferential inhibitors, and the patients had previous history of allergic reaction with these drugs.

In two patients, the diagnosis was accomplished by in vitro tests, namely after positive results in specific IgE to bela-lactams (one patient with specific IgE to amoxicillin, ampicillin and penicillin G and V > 100 kU/L, and the other patient with specific IgE to amoxicillin 11.4 kU/L).

Twenty patients were diagnosed through DPT with the culprit drug. We highlight six patients with positive BL oral challenge (four with amoxicillin, one with clavulanic acid [with negative DPT to amoxicillin] and another with cefuroxime) and one patient who had positive ropivacaine subcutaneous challenge. In the remaining cases the culprit drugs were NSAIDs.

Table II *- Culprit drugs by age group.*

Drug class / Drug	Total (n = 125)	< 18 years (n = 13)	≥ 65 years (n = 8)
NSAIDs	54 (43.2%)	6	3
acetylsalicylic acid	15	1	
ibuprofen	13	3	
metamizole	14	1	2
diclofenac	9		1
paracetamol	3	2	
etodolac	1		
ketorolac	1		
clonixin	1		
Antibiotics	52 (41.6%)	7	5
beta-lactams	43	7	3
amoxicillin	27	6	2
cefazoline	7		
cefuroxime	1		
clavulanic acid	2		
flucloxacillin	2	1	
penicillin	4		1
quinolones ciprofloxacin	4		2
macrolides clarithromycin	3		
fosfomycin	1		
minocycline	1		
Others	21 (16.8%)		
Proton pump inhibitors	6		
omeprazole	3		
pantoprazole	2		
esomeprazole	1		
Neuromuscular blocking drugs	5		
atracurium	3		
rocuronium	1		
succinylcholine	1		
carboplatin	3		
Corticosteroids	2*		
hydrocortisone	2		
budesonide	1		
Local anesthetics	2		
bupivacaine	1		
lidocaine	1		
ranitidine	1		
midazolam	1		
patent blue	1		

* One patient had anaphylaxis to both hydrocortisone and budesonide.

Table III *- Positive results of skin testing (72 patients).*

Positive skin tests	Patients
SPT (n)	16
BL (AX-7, Pen-1)	8
metamizole	4
quinolone (levofloxacin)	1
carboplatine	1
PPI (esomeprazole and omeprazole)	1[1]
local anesthetics (lidocaine, mepivacaine)	1[1]
IDT (n)	59
BL (AX-12, PPL-3, MD-1, cefazoline-7, flucloxacillin-2, clavulanic acid-1)	24[2]
NSAIDs (metamizole-5, diclofenac-4, paracetamol-1)	10
NMBD (atracurium-3, cisatracurium, rocuronium, succinilcoline)	5[3]
PPI (omeprazole-3, pantoprazole-2)	5
macrolides (clarithromycin-3, erythromycin-1)	3[4]
quinolones (ciprofloxacin, levofloxacin)	2
carboplatine	2
hydrocortisone	1
midazolam	1
local anesthetics (bupivacaine, ropivacaine and procaine)	1[1]
ranitidine	1

Abbreviations: AX, amoxicillin; BL, beta-lactams; IDT, intradermal test; MD, sodium benzylpenilloate - minor determinant; NMBD, neuromuscular blocking drugs; Pen, penicillin; PPL, benzylpenicilloyl octa-L-lysine; PPI, proton pump inhibitors; SPT, skin prick test.
Three patients had positive results to both SPT and IDT (different extracts within the same class, quinolones and local anesthetics; and one patient with drugs of different classes, metamizole and penicillin).
[1]Positive skin tests in the same patient; [2]Two patients have IDT positive with both amoxicillin and PPL; [3]One patient had IDT positive with both atracurium and cisatracurium; [4]One patient had IDT positive with both clarithromycin and erythromycin.

In case of anaphylaxis to local anesthetics, both patients were found to be allergic to all drugs within this pharmacological group. It was not possible to find an alternative drug (neither amides nor esters), therefore these two patients were advised to keep strict avoidance of all local anesthetics.
We found seven patients with immediate severe reactions in perioperative setting to cefazolin. One of these patients developed Kounis syndrome after cefazolin infusion. Exploring the cross-reactivity between cefazolin and other BL we confirmed that all these patients with IgE-mediated hypersensitivity reactions to cefazolin can tolerate other beta-lactams, which explains a selective pattern of reactivity.

Discussion

We found that NSAIDs and antibiotics were the most common causes of DIA. However, there is only a slight predominance of NSAIDs (43.2%) as culprit drugs comparing to antibiotics, which were responsible for 41.6% of the anaphylactic reactions. In our sample the anesthetics agents, namely the neuromuscular blocking drugs, were less often reported as culprit of DIA. Anaphylaxis related to local anesthetics are very rare, considering how often they are used, but we had two patients with severe reactions. It is also important to notice the emergence of proton pump inhibitors (six patients), previously considered as unsuspected elicitors of anaphylaxis (13). We did not find any case of severe reaction due to radiocontrast agents. In our sample, we did not register any fatal case of DIA.
Considering an American survey from 1999 to 2010, the antibiotics accounted for 40% of the fatal episodes, mainly penicillins, followed by cephalosporins, sulfonamides and macrolides; radiocontrast agents were implicated in 27% of fatalities and antineoplastic drugs in 12.5%. The remaining culprit drugs

were NSAIDs, serum, opiates, antihypertensive agents, and anesthetic agents (5).

According to a Portuguese drug anaphylaxis survey over a 4-year period (2007-2010), NSAIDs were responsible for 48% of all cases (acetylsalicylic acid, diclofenac, and ibuprofen as main culprits) (6). Similar results were found in a 6-year observation study performed in a Spanish tertiary university hospital, in which NSAIDs were responsible for 49% of the anaphylactic reactions (dipyrone, aspirin and diclofenac as main culprits) (14). Comparing to a previous study reporting a decade review of reactions to a Portuguese Pharmacovigilance Authority, NSAIDs are the culprit drugs in 13% of cases (after antibiotics) (15). In the same study, a subgroup analysis in pediatric population showed that NSAIDs account for 7% of the reported cases (15). According to Online Latin American Survey on Anaphylaxis (OLASA), NSAIDs were the culprit agents in 73% of the DIA (16).

NSAIDs were the main cause of DIA in our sample. It is consensual that COX-1 inhibitors are the main class within the group involved in anaphylaxis. In our study, acetylsalicylic acid, ibuprofen, metamizole and diclofenac account for 87% of all cases related to NSAIDs. We stress three cases with anaphylaxis to paracetamol, two of them at pediatric age, confirmed by DPT. Despite the small number of children and adolescents (13 patients), in this age group antibiotic-induced anaphylaxis (7 patients) is slightly higher than NSAIDs-induced anaphylaxis (6 patients).

Pyrazolones (metamizole) are a common cause of NSAIDs-induced anaphylaxis. Anaphylaxis was reported in 18-30% of patients hypersensitive to pyrazolones (17). In our sample, metamizole accounts for 26% of NSAIDs-induced anaphylaxis.

Regarding antibiotic-induced anaphylaxis, our results agree with previous reports, showing a higher rate of episodes related to beta-lactams (83%) comparing to other groups of non-beta-lactam antibiotics. In a UK database of anaphylaxis, amoxicillin was the most prevalent cause of antibiotic-related anaphylaxis (18). Benzylpenicillin, the initial inducer of allergic reactions, has been replaced by amoxicillin and recently, although in a lesser extent, by cephalosporins. These molecules often show extent cross-reactivity among similar chemical compounds. However, selective responses have been observed, restricted to one group or one single compound, as occurs in the group of cephalosporins (19). In our sample we found 7 patients allergic to cefazolin (16% of beta-lactam-induced anaphylaxis). We speculate this higher incidence might be due to the frequent use of this first-generation cephalosporin, by parenteral route in prophylactic surgical protocols.

The incidence of perioperative anaphylaxis varies between studies from different countries, ranging from 1/1250 to 1/18 600 (20). There is a substantial geographical variability of drugs or substances involved. Studies in different countries have shown that neuromuscular blocking drugs are a leading cause of perioperative anaphylaxis (20-22). Reactions involving antibiotics, dyes, or chlorhexidine become more frequent in most series. Reactions to latex are decreasing, due to effective avoidance measures (20). In our study, neuromuscular blocking drugs were the second most common cause of perioperative anaphylaxis (29.4%) after antibiotics (41.2%). Reactions involving local anesthetics are very uncommon in series from all countries (20). Our series includes one case of anaphylaxis to bupivacaine, whose allergological workup revealed hypersensitivity to all local anesthetics (amides and esters).

Analyzing host related factors, females appear to be more likely to develop drug allergies than males (7). We found similar results, with predominance of cases in females (66%).

Regarding age groups, it is not clearly demonstrated if the incidence of drug allergy is lower in children. We documented only 13 patients aged under 18 years (10%). We can speculate the lower rate of anaphylactic reactions in children and adolescents can be explained by lower exposure and time necessary for sensitization to occur (7).

In our sample, skin tests were useful to confirm IgE-mediated reactions to the suspected drugs and to assess potential cross-reactivity. Etiologic diagnosis of DIA was supported by skin testing in 58% of the patients. Diagnostic accuracy and standard concentrations of most of the skin tests used are widely proven, allowing to avoid a DPT in case of positive result.

Conclusions

NSAIDs and antibiotics were responsible for most cases of DIA. Anaphylactic reactions can be reported at any age. The heterogeneity of mechanisms involved, the severity of clinical reactions and the lack of standardized in vivo and/or in vitro tests do not allow to confirm the diagnosis in all cases. Patients with DIA should be evaluated in specialized centers, to perform accurate diagnosis, to prevent recurrence, and to find safe alternatives.

Conflict of interest

The authors declare that they have no conflict of interest.

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An atlas of IgE sensitization patterns in different Italian areas. A multicenter, cross-sectional study

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Abbreviations
NW, North-West; NE, North-East; C, Centre; S, South; Is, Islands subset.

Summary
Background. *The development of recombinant technology supported the allergy diagnostic work-up in the daily clinical practice, representing a useful tool for epidemiological studies.* ***Methods.*** *An atlas of the IgE sensitization profiles found throughout Italy was prepared from a nationwide, multicenter, cross-sectional study.* ***Results.*** *6052 unselected consecutive individuals, belonging to North-West [NW], North-East [NE], Centre [C], South [S], and Islands subset [Is] were evaluated by means of the ImmunoCAP ISAC test. The top-ranked sensitizations found were Cup a 1 in [C] (58.1%) and [S] (53.6%), Phl p 1 in the North (from 46.1% to 49%), and Cyn d 1 in [Is] (44.2%). High frequency of house dust mite group 2 molecules sensitization was found in [C] (36.9%) and [S] Italy (40.8%), whilst low level of reactivity was recorded in [NW] (20%). Pellitory hypersensitivity was mainly found in [C], [S], and [Is], whilst ragweed Amb a 1 sensitivity was particularly found in [NW] Italy. IgE recognition of PR-10, profilin, and nsLTP was mutually exclusive in 69.1% of cases, PR-10 reactivity mostly occurring in [NE], profilin in [NW], and nsLTP molecules recognition mainly recorded in [C] and [S].* ***Conclusions.*** *Divergent IgE sensitization patterns were found along Italy, possibly linked to the distinct geographical locations, indicating multiplex system IgE analysis as a reliable approach for epidemiological evaluation even in small geographical areas.*

Introduction

Allergic diseases are rising worldwide, affecting about one quarter of the total population in industrialized countries mainly as a consequence of genetic, epigenetic, and environmental factors affecting predisposed individuals (1). In recent years, the development of recombi-nant technology has led to important achievements in allergen characterization, purification and production, thus supporting the allergy diagnostic work-up in the daily clinical practice (2,3). The microarray technology, which allows the simultaneous detection of IgE reactivity towards multiple allergens while using a minimum amount of serum (4), is considered a useful tool for epidemiological studies (5), and in particular for IgE profiling of patients affected by respiratory symptoms (6), atopic dermatitis (7,8), and food allergy (9,10), leading to a more accurate diagnosis of sensitization (11).

It is widely known that allergic sensitization profiles strongly reflect the lifestyle and the environment of the studied population (12). In this regard, several studies have shown that different IgE sensitiza-

tion patterns can be demonstrated, by means of molecular diagnostic approaches, for grasses or tree pollen allergy in different regions of Spain (13,14) or Italy (15,16,17), thus representing possible loco-regional signatures of sensitization. This has also been demonstrated in other countries, such as Africa and Asia (18,19,20).

Italy, located in South-Central Europe, includes a boot-shaped peninsula and two large islands, i.e. Sardinia and Sicily. The Alps form the northern boundary, and the Apennine mountain range runs along the entire peninsula, with the Po river valley, the largest plain found in Italy, placed between them. Due to such geographical characteristics, Italy has a multiplicity of climate systems. Following the Köppen-Geiger classification system, the most widely used climate categorization scheme, the North-East of Italy, the Po and the Adige valleys show a "Humid subtropical climate [Cfa]", a "Mediterranean climate [Csa]" involves all the coastal areas, whilst a "Mediterranean mild climate [Csb]" is found in-land and in Southern Italy. A number of other microclimates can be found in Italy, such as an "Oceanic climate [Cfb]" in the Apennines, a "Humid continental climate [Dfb]" in the Alps (but above the tree line a "Tundra climate" is registered), and a "Cold continental climate [Dfc]" in the alpine valleys. Some regions in Italy have a type of weather presenting elements belonging to both Cfa and Csa, and therefore cannot be uniquely classified. Such climatic variety largely affects the vegetation, thus allowing distinct types of cultivation, e.g. olive trees, in some regions and not in other ones. Such characteristics represent a wonderful model to study the impact of climate and geographical location on IgE sensitization.

In this light, we present a multicenter study designed to comprehensively analyze the IgE sensitization profile evaluated by means of multiplex testing performed during routine clinical care, in samples from different regions throughout Italy, to verify the impact of geographical and climatic characteristics on the distribution of allergic sensitization.

Methods

Patients

Six allergy units (Florence, Milan, Palermo, Pavia, Pordenone, and Rome) distributed in different Italian areas participated to this multicenter study. Between September 2015 and August 2016, doctors enrolled unselected patients presenting with a history of at least one of the following: adverse reactions to foods, allergic rhinitis, bronchial asthma, and/or atopic eczema. Blood samples were taken from all patients; after centrifugation, properly coded sera were kept at -20 °C until their use for in-vitro analyses.

All ISAC tests were performed during routine care, and the samples were anonymized, so that no personal data, with the exception of age and sex, was available. The Institutional Review Board of IDI-IRCCS confirmed that an ethical approval was not required (n. 493.1).

IgE antibody measurements

Serum IgE reactivity was analyzed using the ISAC platform (Thermo Fisher Scientific, Uppsala, Sweden) by means of the latest commercially available ImmunoCAP-ISAC as per manufacturer's instructions. The ISAC microarray in the current versions is a fully commercial product. Allergens spotted on the chip are determined by the manufacturer, and presently this mainly depends on molecule availability from a number of different providers, i.e. either companies or research labs.

In brief, ImmunoCAP-ISAC 112 slides were washed, rinsed and dried at room temperature (RT). Undiluted serum (30 µl) from each patient was pipetted on to the slide, and after 120 min incubation at RT in a humid chamber, slides were washed, rinsed and dried. IgE binding was detected by addition of an anti-human secondary antibody (Thermo Fisher Scientific). Slides were then washed, rinsed, dried, and stored in the dark until scanning. Images were acquired by scanning allergen biochips with a CapitalBio LuxScan® 10K microarray scanner. IgE values were expressed as ISU arbitrary units (ISAC Standardized Units), corresponding to IgE antibody levels in the ng/ml range (detection limit: 0.01 ISU-E) (21-23).

Statistics

All data were analyzed data using the IBM SPSS Statistics, version 21 statistical package (Armonk, NY, USA) for statistical evaluation. The TD-Synergy Laboratory Information System was used to search and collect demographic information, i.e. age and gender, and clinical and laboratory data for Allergy Clinic patients who attended the outpatient Allergy clinic and underwent specific IgE testing.

Categorical variables were analyzed using the Pearsons' χ^2 or Fisher's exact test. Differences between prevalence were evaluated using the nonparametric Mann-Whitney U-test. The degree of relationship between the quantitative variables studied was analyzed using the Pearson Correlation (r) coefficient. Statistical significance cut-off level has been set for $p < 0.05$. The degree of relationship between quantitative variables was analyzed using the Spearman's correlation (r) coefficient, given the non-parametric distribution of the observed values.

Separate modelling was performed for each condition including all molecules, in addition to sex and age. P values < 0.05 were considered significant.

Result

Study cohort

A total of 6052 ISAC-positive adult individuals (53.3% female; mean age 33 ± 19 years) were recruited by the participating centers. Patients were subdivided according to the classification of terri-

torial units for statistics, (NUTS, for the French *Nomenclature d'Unités Territoriales Statistiques*), i.e. a geocode standard developed by the European Union for referencing the administrative divisions of Italy and other countries for statistical purposes. Briefly, patients were assigned to one of the following macro-regions: (i) 1217 participants from the Aosta Valley, Liguria, Lombardy, and Piedmont were grouped in the North-West [NW], (ii) 1450 participants from Emilia-Romagna, Friuli-Venezia Giulia, Trentino-South Tyrol, and Veneto in the North-East [NE], (iii) 2332 participants from Lazio, Marches, Tuscany, and Umbria in the Centre [C], (iv) 802 participants from Abruzzo, Apulia, Basilicata, Calabria, Campania, and Molise in the South [S], and (v) 251 participants from Sardinia and Sicily in the Islands subset [Is].

Prevalence of allergen IgE recognition

Table I shows the proportion of the first twenty positive allergens found in the five macro-regions. Cypress pollen allergen Cup a 1 was the most common sensitization in C and S Italy, whilst the timothy grass major-pollen allergen Phl p 1, and its homologous bermuda grass allergen, Cyn d 1, were the most prevalent in the other macro-regions. In Centre and South Italy, cypress pollen and grass pollen sensitizations were followed by the house dust mite allergen Der f 2, in Is by pellitory Par j 2, by birch pollen Bet v 1 in NE, and by cat uteroglobin (Fel d 1) in NW. Interestingly, the peach lipid transfer protein (LTP) Pru p 3 was the most common food allergen in C, S and Is, whilst PR10 molecules were the most frequent food allergens found in NE and NW. Profilin allergens and the major ragweed pollen allergen, Amb a 1, were included among the first twenty positive allergens only in NW. No difference in cross-reactive carbohydrate determinants (CCD) IgE recognition was observed throughout Italy, with a prevalence ranging from 9.8% to 12.7% of the IgE sensitized participants.

Table I. *Frequency of IgE sensitization to the top ranked 20 allergenic molecules tested on ISAC-112 microarray in Italy macro-regions. Prevalence have been calculated on the respective tested subjects positive to at least one of the 112 allergens on the microarray.*

	NE		**NW**		**C**		**S**		**Is**	
	n= 1450		**n= 1217**		**n= 2332**		**n= 802**		**n= 251**	
1	Phl p 1	46,1%	Phl p 1	49,0%	Cup a 1	58,1%	Cup a 1	53,6%	Cyn d 1	44,2%
2	Cyn d 1	41,9%	Cyn d 1	44,9%	Phl p 1	54,6%	Phl p 1	47,1%	Cup a 1	41,0%
3	Cup a 1	35,4%	Phl p 5	33,9%	Cyn d 1	48,6%	Cyn d 1	45,4%	Phl p 1	38,2%
4	Bet v 1	32,8%	Phl p 4	30,9%	Cry j 1	41,4%	Der f 2	40,8%	Par j 2	28,7%
5	Phl p 5	31,6%	Fel d 1	26,8%	Der f 2	35,9%	Der p 2	39,0%	Fel d 1	28,3%
6	Fel d 1	31,5%	Amb a 1	26,7%	Der p 2	35,8%	Cry j 1	38,2%	Cry j 1	27,9%
7	Der f 2	30,9%	Phl p 2	25,7%	Phlp 4	34,8%	Par j 2	35,7%	Der f 2	27,9%
8	Der p 2	29,9%	Phl p 6	25,1%	Fel d 1	32,4%	Der f 1	31,2%	Der p 2	25,5%
9	Cor a 1.01	28,3%	Bet v 1	23,9%	Phl p 5	31,9%	Der p 1	30,3%	Ole e 1	25,5%
10	Mal d 1	28,1%	Cor a 1.01	22,1%	Der f 1	31,6%	Phl p 4	29,9%	Der f 1	24,7%
11	Aln g 1	27,7%	Ole e 1	21,5%	Der p 1	30,9%	Ole e 1	28,1%	Der p 1	23,9%
12	Phl p 4	27,6%	Cor a 1.04	20,8%	Par j 2	27,9%	Pru p 3	27,2%	Phl p 4	22,7%
13	Cor a 1.04	26,6%	Mal d 1	20,7%	Phl p 2	27,5%	Fel d 1	26,6%	Pru p 3	22,3%
14	Der p 1	25,7%	Der p 2	20,0%	Ole e 1	27,5%	Phl p 5	26,1%	Jug r 3	18,3%
15	Der f 1	25,6%	Aln g 1	19,9%	Phl p 6	22,7%	Jug r 3	24,3%	Pla a 2	17,9%
16	Pru p 1	24,1%	Der f 2	19,7%	Pru p 3	21,3%	Pla a 3	22,4%	Phl p 5	15,5%
17	Cry j 1	23,7%	Pru p 1	19,2%	Pla a 2	18,5%	Ara h 9	20,1%	Pla a 3	15,5%
18	Ole e 1	23,4%	Cup a 1	18,7%	Jug r 3	18,3%	Phl p 2	19,6%	Art v 3	13,5%
19	Phl p 2	22,2%	Hev b 8	18,2%	Pla a 3	16,5%	Art v 3	19,5%	Phl p 2	13,1%
20	Phl p 6	16,4%	Mer a 1	18,0%	Phl p 11	15,5%	Phl p 6	19,1%	Alt a 1	15,5%

A. Species specific molecules

1. Grass pollen allergy

Table II reports proportion for each of the grass pollen allergens in the different areas. Overall, patients from Central Italy showed a higher reactivity compared to the other Italian regions, whereas a higher occurrence of profilin, Phl p 12, reactivity was recorded in NW Italy. Interestingly, Cyn d 1 IgE recognition overcame Phl p 1 recognition in Is, whilst in the other regions Phl p 1 reactivity was always highly prevalent. No difference in the occurrence of polcalcin, Phl p 7, sensitization was recorded throughout Italy.

2. House dust mite allergy

House dust mite sensitization was higher in C and S Italy, particularly the group 2 allergens (**table II**). Interestingly, the highest level of Lep d 2 was recorded in the South. Der p 10 reactivity was rarely observed in the NW part of the country. No difference in Blo t 5 prevalence sensitization was recorded throughout Italy.

3. Tree pollen allergy

A higher reactivity to *Fagales* was detected in the North (particularly in NE), similar to the sensitization profile of central Europe (**table II**). On the other hand, cypress pollen sensitization was most prevalent in C and S Italy. Olive and plane tree reactivity was highest in C.

4. Weed pollen allergy

Pellitory was one of the most important causes of IgE sensitization in S, C, and I, whereas it was rarely observed in the

Table II *- Prevalence of IgE sensitization to Inhalant allergens. Results are based on two-sided tests assuming equal variances with significance level 0.01. For each significant pair, the key of the smaller category appears under the category with larger mean.*

	A Centre	B Isles	C Nord-East	D Nord-West	E South
Grasses					
Cyn d 1	*1133* 48,6% C	*111* 44,2%	*607* 41,9%	*546* 44,9%	*364* 45,4%
Phl p 1	*1274* 54,6% BCDE	*96* 38,2%	*669* 46,1%	*596* 49,0% B	*378* 47,1%
Phl p 2	*642* 27,5% BCE	*33* 13,1%	*322* 22,2% B	*313* 25,7% BE	*157* 19,6%
Phl p 4	*812* 34,8% BC	*57* 22,7%	*400* 27,6%	*376* 30,9%	*240* 29,9%
ıl p 5.0204	*743* 31,9% BE	*39* 15,5%	*458* 31,6% B	*412* 33,9% BE	*209* 26,1% B
Phl p 6	*529* 22,7% BC	*22* 8,8%	*238* 16,4% B	*306* 25,1% BCE	*153* 19,1% B
Phl p 7	*82* 3,5%	*5* 2,0%	*48* 3,3%	*34* 2,8%	*25* 3,1%
Phl p 11	*362* 15,5% B	*16* 6,4%	*196* 13,5% B	*186* 15,3% B	*97* 12,1%
Phl p 12	*243* 10,4%	*14* 5,6%	*119* 8,2%	*172* 14,1% ABCE	*77* 9,6%
House Dust Mite					
Der f 1	*738* 31,6% CD	*62* 24,7% D	*371* 25,6% D	*184* 15,1%	*250* 31,2% CD
Der p 1	*721* 30,9% CD	*60* 23,9% D	*373* 25,7% D	*182* 15,0%	*243* 30,3% D
Der f 2	*838* 35,9% CD	*70* 27,9% D	*448* 30,9% D	*240* 19,7%	*327* 40,8% BCD
Der p 2	*836* 35,8% BCD	*64* 25,5%	*434* 29,9% D	*243* 20,0%	*313* 39,0% BCD
Lep d 2	*252* 10,8% D	*24* 9,6% D	*131* 9,0% D	*60* 4,9%	*122* 15,2% ACD
Der p 10	*133* 5,7% D	*11* 4,4%	*93* 6,4% D	*21* 1,7%	*62* 7,7% D
Blo t 5	*138* 5,9%	*10* 4,0%	*73* 5,0%	*60* 4,9%	*59* 7,4%

	A Centre	B Isles	C Nord-East	D Nord-West	E South
Tree pollen allergens					
Aln g 1	*181* 7,8%	*8* 3,2%	*401* 27,7% ABDE	*242* 19,9% ABE	*79* 9,9% B
Bet v 1	*268* 11,5%	*15* 6,0%	*475* 32,8% ABDE	*291* 23,9% ABE	*104* 13,0% B
Cor a 1.0101	*261* 11,2% B	*12* 4,8%	*410* 28,3% ABDE	*269* 22,1% ABE	*101* 12,6% B
Cry j 1	*965* 41,4% BCD	*70* 27,9% D	*343* 23,7% D	*192* 15,8%	*306* 38,2% BCD
Cup a 1	*1355* 58,1% BCD	*103* 41,0% D	*513* 35,4% D	*227* 18,7%	*430* 53,6% BCD
Ole e 1	*641* 27,5% D	*64* 25,5%	*340* 23,4%	*262* 21,5%	*225* 28,1% D
Ole e 7	*168* 7,2% CD	*17* 6,8%	*60* 4,1%	*44* 3,6%	*92* 11,5% ACD
Ole e 9	*91* 3,9%	*6* 2,4%	*50* 3,4%	*27* 2,2%	*41* 5,1% D
Pla a 1	*74* 3,2% CD	*3* 1,2%	*9* 0,6%	*12* 1,0%	*11* 1,4%
Pla a 2	*431* 18,5% CD	*45* 17,9% D	*182* 12,6%	*133* 10,9%	*133* 16,6% D
Pla a 3	*384* 16,5% CD	*39* 15,5% CD	*121* 8,3%	*100* 8,2%	*180* 22,4% ACD
Weed pollen allergens					
Amb a 1	*28* 1,2%	*6* 2,4%	*79* 5,4% AE	*325* 26,7% ABCE	*11* 1,4%
Art v 1	*131* 5,6%	*10* 4,0%	*169* 11,7% AB	*112* 9,2% A	*69* 8,6% A
Art v 3	*284* 12,2% CD	*34* 13,5% D	*128* 8,8%	*79* 6,5%	*156* 19,5% ACD
Par j 2	*650* 27,9% CD	*72* 28,7% CD	*77* 5,3%	*140* 11,5% C	*286* 35,7% ACD
Pla l 1	*167* 7,2% B	*4* 1,6%	*198* 13,7% ABDE	*61* 5,0%	*39* 4,9%

	A Centre	B Isles	C Nord-East	D Nord-West	E South
Animal allergens					
Can f 1	*234* 10,0% BD	*9* 3,6%	*155* 10,7% BD	*78* 6,4%	*73* 9,1% B
Can f 2	*61* 2,6%	*2* 0,8%	*58* 4,0% D	*20* 1,6%	*22* 2,7%
Can f 3	*55* 2,4%	*2* 0,8%	*48* 3,3% D	*19* 1,6%	*21* 2,6%
Can f 5	*291* 12,5%	*20* 8,0%	*198* 13,7%	*123* 10,1%	*94* 11,7%
Equ c 1	*112* 4,8% D	*5* 2,0%	*64* 4,4%	*32* 2,6%	*36* 4,5%
Equ c 3	*37* 1,6%	*2* 0,8%	*33* 2,3% D	*10* 0,8%	*12* 1,5%
Fel d 1	*756* 32,4% DE	*71* 28,3%	*457* 31,5%	*326* 26,8%	*213* 26,6%
Fel d 2	*69* 3,0%	*3* 1,2%	*53* 3,7% D	*21* 1,7%	*23* 2,9%
Fel d 4	*100* 4,3%	*4* 1,6%	*71* 4,9%	*37* 3,0%	*34* 4,2%
Mus m 1	*54* 2,3%	*3* 1,2%	*66* 4,6% AD	*19* 1,6%	*22* 2,7%
Latex allergens					
Hev b 1	*15* 0,6%	*1* 0,4%	*9* 0,6%	*12* 1,0%	*6* 0,7%
Hev b 3	*19* 0,8%	*1* 0,4%	*12* 0,8%	*8* 0,7%	*6* 0,7%
Hev b 5	*15* 0,6%	*0* 0,0%	*19* 1,3%	*12* 1,0%	*6* 0,7%
Hev b 6.01	*55* 2,4%	*3* 1,2%	*42* 2,9%	*16* 1,3%	*16* 2,0%
Hev b 8.0204	*291* 12,5%	*22* 8,8%	*182* 12,6%	*221* 18,2% ABCE	*92* 11,5%

Table III *- Prevalence of IgE sensitization to Pan-Allergens. Results are based on two-sided tests assuming equal variances with significance level 0.01. For each significant pair, the key of the smaller category appears under the category with larger mean.*

	A Centre	B Isles	C North-East	D North-West	E South
PR-10 allergens					
Act d 8	*52* 2,2%	*4* 1,6%	*70* 4,8% A	*62* 5,1% A	*22* 2,7%
Aln g 1	*181* 7,8%	*8* 3,2%	*401* 27,7% ABDE	*242* 19,9% ABE	*79* 9,9% B
Api g 1	*59* 2,5%	*2* 0,8%	*129* 8,9% ABE	*82* 6,7% ABE	*25* 3,1%
Ara h 8	*105* 4,5%	*6* 2,4%	*197* 13,6% ABE	*146* 12,0% ABE	*48* 6,0%
Bet v 1	*268* 11,5%	*15* 6,0%	*475* 32,8% ABDE	*291* 23,9% ABE	*104* 13,0% B
Cor a 1.0101	*261* 11,2% B	*12* 4,8%	*410* 28,3% ABDE	*269* 22,1% ABE	*101* 12,6% B
Cor a 1.0401	*207* 8,9%	*10* 4,0%	*385* 26,6% ABDE	*253* 20,8% ABE	*77* 9,6% B
Gly m 4	*85* 3,6%	*3* 1,2%	*172* 11,9% ABE	*116* 9,5% ABE	*36* 4,5%
Mal d 1	*222* 9,5%	*12* 4,8%	*407* 28,1% ABDE	*252* 20,7% ABE	*87* 10,8% B
Pru p 1	*173* 7,4%	*9* 3,6%	*350* 24,1% ABDE	*234* 19,2% ABE	*78* 9,7% B
Lipocalin					
Can f 1	*234* 10,0% BD	*9* 3,6%	*155* 10,7% BD	*78* 6,4%	*73* 9,1% B
Can f 2	*61* 2,6%	*2* 0,8%	*58* 4,0% D	*20* 1,6%	*22* 2,7%
Equ c 1	*112* 4,8% D	*5* 2,0%	*64* 4,4%	*32* 2,6%	*36* 4,5%
Fel d 4	*100* 4,3%	*4* 1,6%	*71* 4,9%	*37* 3,0%	*34* 4,2%
Mus m 1	*54* 2,3%	*3* 1,2%	*66* 4,6% AD	*19* 1,6%	*22* 2,7%
Polcalcin					
Bet v 4	*70* 3,0%	*5* 2,0%	*41* 2,8%	*28* 2,3%	*18* 2,2%
Phl p 7	*82* 3,5%	*5* 2,0%	*48* 3,3%	*34* 2,8%	*25* 3,1%

	A Centre	B Isles	C North-East	D North-West	E South
Lipid Transfer Proteins (nsLTPs)					
Ara h 9	*333* 14,3% CD	*31* 12,4% C	*103* 7,1%	*100* 8,2%	*161* 20,1% ACD
Art v 3	*284* 12,2% CD	*34* 13,5% D	*128* 8,8%	*79* 6,5%	*156* 19,5% ACD
Cor a 8	*265* 11,4% CD	*28* 11,2% C	*77* 5,3%	*87* 7,1%	*133* 16,6% ACD
Jug r 3	*426* 18,3% CD	*46* 18,3% CD	*124* 8,6%	*118* 9,7%	*195* 24,3% ACD
Ole e 7	*168* 7,2% CD	*17* 6,8%	*60* 4,1%	*44* 3,6%	*92* 11,5% ACD
Pla a 3	*384* 16,5% CD	*39* 15,5% CD	*121* 8,3%	*100* 8,2%	*180* 22,4% ACD
Pru p 3	*497* 21,3% CD	*56* 22,3% CD	*157* 10,8%	*140* 11,5%	*218* 27,2% ACD
Tri a 14	*121* 5,2% D	*8* 3,2%	*50* 3,4%	*35* 2,9%	*60* 7,5% CD
Profilin allergens					
Bet v 2	*255* 10,9%	*16* 6,4%	*168* 11,6%	*210* 17,3% ABCE	*87* 10,8%
Hev b 8.0204	*291* 12,5%	*22* 8,8%	*182* 12,6%	*221* 18,2% ABCE	*92* 11,5%
Mer a 1	*287* 12,3% B	*13* 5,2%	*175* 12,1% B	*219* 18,0% ABCE	*95* 11,8% B
Phl p 12	*243* 10,4%	*14* 5,6%	*119* 8,2%	*172* 14,1% ABCE	*77* 9,6%
Tropomyosin allergens					
Ani s 3	*88* 3,8% D	*9* 3,6%	*79* 5,4% D	*16* 1,3%	*42* 5,2% D
Bla g 7	*89* 3,8% D	*9* 3,6%	*111* 7,7% AD	*17* 1,4%	*41* 5,1% D
Der p 10	*133* 5,7% D	*11* 4,4%	*93* 6,4% D	*21* 1,7%	*62* 7,7% D
Pen m 1	*124* 5,3% D	*14* 5,6% D	*77* 5,3% D	*19* 1,6%	*62* 7,7% D

North, particularly in NE (**table II**). Hypersensitivity to Amb a 1, the major ragweed pollen allergen, was highest in NW Italy, whilst it was rarely observed in Central and Southern Italy. The major mugwort pollen allergen, Art v 1 was frequent in NE, whilst the nsLTP Art v 3 showed a different behavior (see LTP section) with a higher incidence in C and S. Pla l 1 reactivity, from plantain (*Plantago lanceolata*) was largely found in North-East Italy.

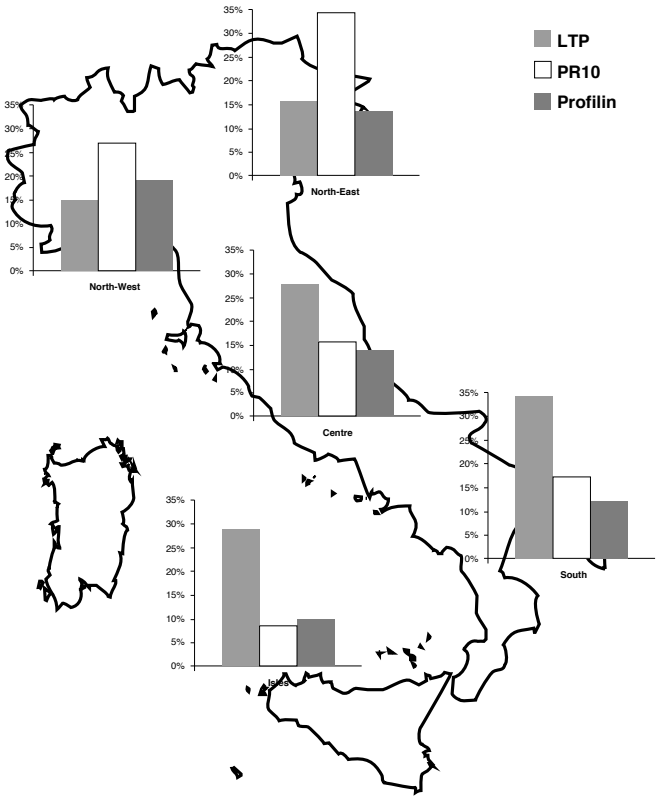
5. Cat, dog and horse
No differences in the prevalence of sensitization to animal dander allergens were observed, with the exception of a significant higher occurrence of Can f 1 reactivity in C, S and NE (**table II**).

6. Latex
No differences in latex molecules reactivity were observed throughout Italy, whilst the reactivity to the profilin, Hev b 8, was mainly found in NW (**table II**) according to the other members of that superfamily (see above).

B. Cross reactive molecules
The overall distribution of profilin, PR-10, nsLTP and tropomyosin prevalence throughout Italy is shown in **figure 1**.

1. Plant food panallergens
Overall, plant food panallergens reactivity was found in 2935 (48.5%) participants. The IgE recognition of PR-10, profilin, and nsLTP was mutually exclusive in 69.1% of cases, ranging from 65% in NE to 83% in the Islands.

Figure 1 *- Pollen food panallergens distributionin Italy macro-regions. Prevalence have been calculated on the respective tested subjects (2332 in C, 251 in I, 1450 in NE, 1217 in NW and 802 in S)*



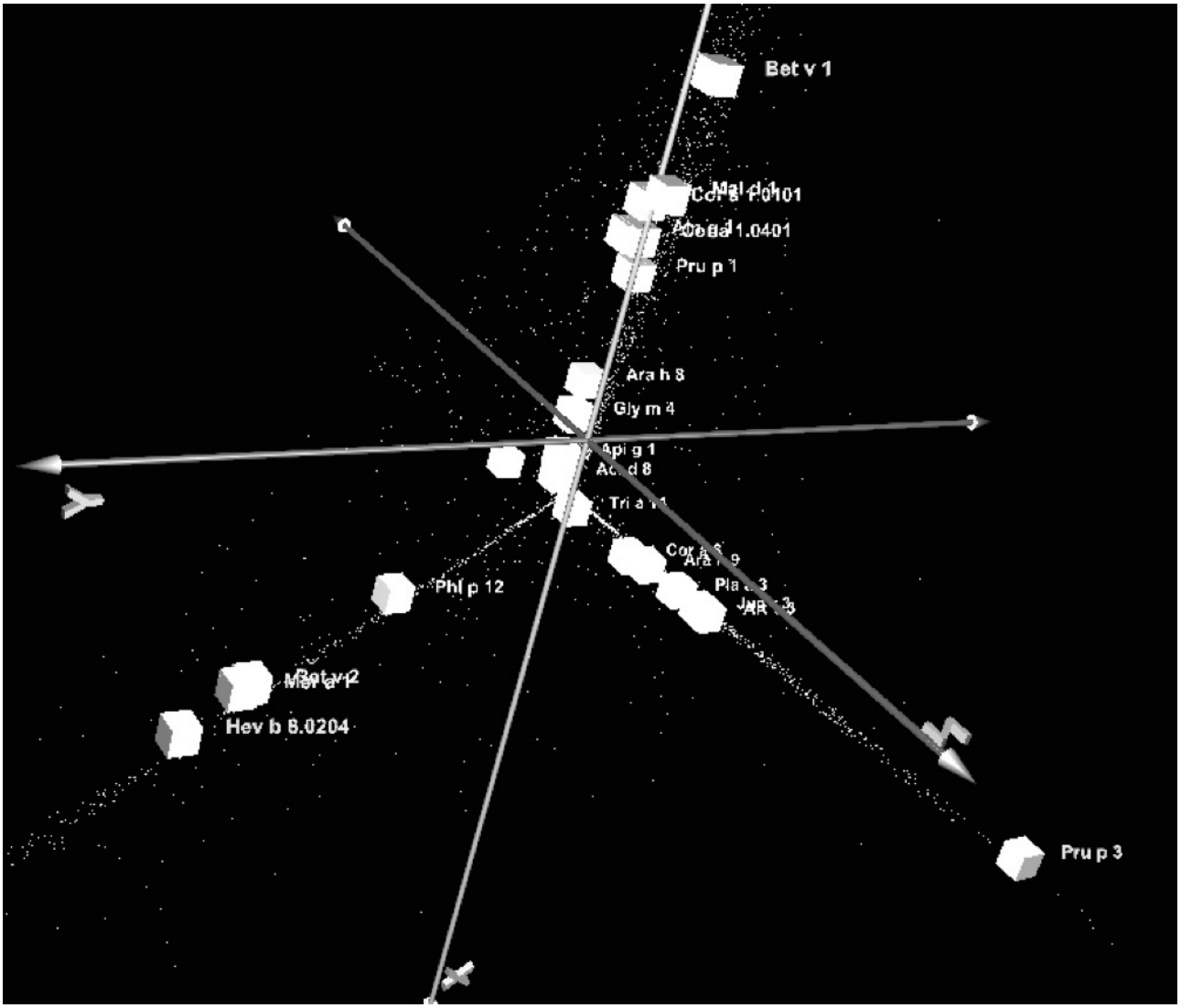
Profilin reactivity affected around 10% of the entire allergic population throughout Italy (**table III**), but in NW the frequency was double than in all other regions (**figure 1**). Interestingly, profilin from latex, Hev b 8, was the top ranked molecule in all macro-regions but the S, where the annual mercury profilin, Mer a 1, was the most prevalent.
In Northern Italy, particularly in the NE, PR-10 reactivity was frequent, with Bet v 1 recognition levels from two to three times higher than in the rest of Italy (**table III**). Accordingly, all the molecules belonging to the PR-10 family showed a comparable consistent pattern.
nsLTP molecules recognition showed an opposite trend if compared to PR-10 (**table III**), prevailing in Center, Islands and, particularly, in the South of Italy where Prt p 3 recognition affected around 1/3 of the entire allergic population (**figure 1**).
Figure 2 recapitulates the results of the principal correspondence analysis, showing three-dimensionally the mutual relationship of Plant food panallergens in terms of IgE co-recognition. Since this statistical approach places variables in a tridimensional space based on their levels of reciprocal relationship (i.e., highly correlated variables are close to each other, while uncorrelated variables are placed far away from each other), we observe here the significantly divergent behavior between PR-10, profilin and LTP molecules in 69% of participants, suggesting the presence of a mutual exclusive IgE recognition in the majority of cases.

2. Tropomyosins, polcalcin, and lipocalins
Similar levels of tropomyosin recognition were recorded throughout Italy, with the exception of NW where such reactivity was rarely observed (**table III**). Der p 10 was the tropomyosin most frequent in C and S, whilst Bla g 7 was the top ranked in NE and Pen m 1 was most common in Is and S. No, or scant differences in the recognition proportion of polcalcin and lipocalins were recorded (**table III**), thus representing sensitization affecting a restricted subset of patients.

Discussion

We studied, by means of a microarray system, the relative frequency of IgE sensitization in several macro-regions in Italy.
Extreme heterogeneity in the IgE sensitization profile was found across Italy, largely depending on the geo-climatic features and the distribution of the allergenic pollen. On the bases of these two criteria, a map of exposure to pollen derived allergens was recently published (24). An increasing body of evidences shows that other factors might play a role in increasing the risk of sensitization and allergy to pollen allergens, including air pollution and climate change, all of them comprised in the new concept of "exposome" (25).

Figure 2 *- Multiple correspondence analysis plot, showing the mutual inter-relationships among plant-food panallergens (each molecule is represented by one dot) in term of IgE reactivity. The plot illustrates the divergent behavior of IgE recognition between PR-10, Profilin and nsLTP.*



Overall, the most frequent cause of sensitization found in Italy was the cypress pollen in C and S (26), and the grass pollen in NE and NW (15). Only in the main Islands, the Bermuda grass (*Cynodon dactylon*) sensitization surpassed timothy grass pollen IgE recognition, thus suggesting differences in the plant distribution or of other local genetic and/or environmental-related factors affecting the IgE mediated response to the allergen. No difference in CCD reactivity was registered throughout Italy.

The group 1 grass allergens always overcame the other grass pollen molecules sensitization across the entire country, thus confirming the possible role of Phl p 1 as the initiator of grass pollen reactivity (27) and its role as genuine marker of grass sensitization in Europe (28).

Par j 2 reactivity was found more often in C, S, and I, but it was virtually absent in the North (29), where birch pollen Bet v 1 and, consequently, the PR-10 related molecules, were among the most common elicitors of sensitization, also as food allergen. On the other hand, the peach nsLTP, Pru p 3, was the most important food allergen found in C, I, and, particularly, in the S, where about 1/3 of the entire population studied was affected, therefore confirming previous observation regarding the relevance of nsLTP sensitization in the Mediterranean area (17).

A surprising low level of house dust mite reactivity was recorded in NW, if compared to the rest of Italy, probably because of the climatic differences, the type of buildings, or a higher prevalence of other HDM allergens like Der p 23.
The high occurrence of ragweed Amb a 1 IgE recognition represented a main feature of the NW sensitization profile, consistently with the area of ragweed distribution in Italy (30).
Cypress and *Fagales* pollen reactivity showed a divergent behavior, the first being mainly distributed in C and S (31) and the former in NE and NW, accordingly with the areas of distribution of these plants and of their pollen (32).
Reactivity for the major allergen of olive tree, Ole e 1, was evenly distributed across Italy, but the putative nsLTP, Ole e 7, was mainly found in the S, similarly to what observed in Spain (33), but probably also related to the higher frequency of nsLTP sensitization observed in the Southern areas of Italy (34,17).
No geographical variability influence was registered for latex sensitization, confirming that such reactivity is apparently not related to environmental exposure.
A particular observation in this study was the radically diverse profile of panallergen sensitization found throughout Italy (**figure 1**): nsLTP reactivity was prevalently found in C and S where the source of sensitization is food, as also suggested by the higher prevalence of IgEs to nsLTP Art v 3 than Art v 1 (the major mugwort allergen) in those regions, compared to the opposite results found in the NE and NW (**table II**). Profilin sensitization was higher in NW and PR10 IgE recognition in the North, particularly in NE, possibly because of the high exposure to the grass profilin and birch PR-10 (Bet v 1), respectively. A mutual exclusive IgE recognition of the three plant food panallergens studied was observed in large part of our Italian cohort. More than 69% percent of patients, in fact, produced specific IgE to a group of plant food panallergens but not to the others. Polcalcin reactivity was equally distributed along the entire Peninsula. Tropomyosin was rarely found among the most common causes of food allergy in NW, where sensitization to the major HDM allergens was low as well. This finding suggests that in that area the major source of sensitization to tropomyosin is HDM, and not crustaceans.
In summary, the present study provides a clear picture of how geographical location could influence sensitization profiles and their clinical expression, even in comparatively small geographical areas.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Restoration of aspirin tolerance following omalizumab treatment in a patient with chronic spontaneous urticaria

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Keywords
chronic spontaneous urticaria; omalizumab; NSAID; aspirin; drug allergy

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Summary
Up to 30% of cases of chronic spontaneous urticaria (CSU) are exacerbated by COX-1 inhibiting nonsteroidal anti-inflammatory drugs (NSAID); this clinical picture is termed NECD (NSAID-exacerbated cutaneous disease). On the other hand, multiple NSAID hypersensitivity may occur in the absence of an underlying CSU also, a situation that is termed NIUA (NSAID-induced urticaria / angioedema).
The present study reports a case of multiple NSAID hypersensitivity that occurred in a man much before he developed severe CSU. Omalizumab treatment eventually induced a remission of the cutaneous disease which was associated with aspirin tolerance, as assessed by open oral challenge with the drug. Altogether, this case suggests that it might be worth to investigate tolerance to aspirin or other strong COX-1 inhibitors in NECD patients showing a complete response to omalizumab, and maybe also the effects of omalizumab in NIUA patients as well.

Introduction

Chronic spontaneous urticaria (CSU) is a rather frequent disorder characterized by the recurrent occurrence of itchy wheals, in many cases associated with angioedema, for more than 6 weeks (1). The disease affects up to 1% of the general population and may severely impair the quality of life (2,3). In up to 30% of cases this condition is associated with an intolerance to nonsteroidal anti-inflammatory drugs (NSAID) which inhibit cyclooxygenase 1 (COX-1) (4). The intake of these drugs during the phases of clinical activity of the underlying skin disease leads to severe exacerbations that may pose the patient at risk of involvement of the upper respiratory tract (5). There are data suggesting that NSAID-hypersensitivity accompanies preferentially severe CSU (6), and that intolerance to NSAID reactivity might precede by years the onset of the spontaneous skin disease (7).
In recent years the monoclonal anti-IgE antibody, omalizumab, has been introduced for the treatment of patients with severe CSU not responding to second generation antihistamines given even at higher than licensed doses, with excellent results in the majority of cases (8). The potentially beneficial effect of omalizumab treatment on NSAID hypersensitivity associated to CSU has not been evaluated so far, and will be considered in the present report.

Case report

A 65-year-old man presented at this allergy department with a 25-year-long history of acute, generalized urticaria/angioedema following the ingestion of chemically distinct NSAID (aspirin, metamizol, diclofenac, and paracetamol) taken in different occasions; the only analgesic drug that he was able to tolerate was the opioid tramadol. Adverse reactions occurred about 15-30 minutes after taking the offending drug. Further, during the

last one and a half year the man had been suffering from severe chronic spontaneous urticaria. The disease did not respond to second generation antihistamines at doses 3 times higher than the licensed dosage and the patient had to take 32 mg/day of methyl-prednisolone orally to control it. An autologous serum skin test (ASST) carried out in another hospital about 6 months before had scored frankly positive.

Baseline lab investigations showed elevated D-dimer plasma levels (1815 ng/ml; n.v. < 500) as well as elevated CRP (11.4 mg/100 ml; n.v. < 0.5), whereas thyroid autoantibodies were negative and ESR was normal.

Therapy with omalizumab 300 mg/month was started, and the patient began to respond after the 3rd administration with a gradual drop of both D-dimer (657 ng/ml) and CRP (0.99 mg/100ml) associated with a reduction of wheals and angioedema. A complete remission (i.e., virtual absence of wheals while taking cetirizine at licensed dose) was eventually obtained only after the administration of the 13th omalizumab course, while temporary withdrawals of the treatment as prescribed by the Italian NHS (the Italian legislation imposes to stop the treatment for at least 2 months after 6 administrations, and to resume it for further 5 administrations only in case of a relapse) were followed by relapses of the disease. After 16 courses of anti-IgE, D-dimer plasma levels as well as CRP were normal (466 ng/ml and < 0.5 mg/100 ml, respectively). Based on the timing of response to anti-IgE, the patient was classified as a "late responder" to the drug (9).

At this point, the patient asked for a possible re-introduction of NSAID in order to treat recurrent joint pain, and accepted (subscribing a written informed consent) to undergo an oral challenge with aspirin in order to check its tolerability. The oral challenge was performed in the clinic under medical supervision. Increasing doses of aspirin were given 1 hour apart up to a normal dose of 500 mg (50 mg + 200 mg + 250 mg) in an open fashion. The patient was instructed and recommended to alert the personnel immediately in case of itching or discomfort; further, 60 minutes after each dose the patient underwent clinical examination looking for wheals and/or angioedema. The patient was kept under control for 1.5 hours after the last provocative dose. No reaction was noted, and the patient reported only a significant reduction of his orthopedic pain. It was concluded that aspirin was tolerated at this point.

Discussion

Recent studies from our group showed that serial measurements of D-dimer plasma levels are a simple and sensitive way to monitor disease activity in patients with chronic spontaneous urticaria (10). The mechanism of action of omalizumab in chronic spontaneous urticaria is still poorly understood. It has been hypothesized that early responders might have circulating IgE specific for self-antigens (11), while a late response is associated with an autoreactive/autoimmune disease, as shown by positive ASST (9). This patient was clearly a late responder. Further, he had a history of multiple NSAID hypersensitivity, that appeared well before the onset of spontaneous chronic urticaria. Thus, based on recent EAACI nomenclature (4), this patient had a NIUA (Nonsteroidal anti-inflammatory drugs-induced urticaria/angioedema). It is impossible to know whether, after the appearance of chronic spontaneous urticaria, he became a case of NECD (Nonsteroidal anti-inflammatory drugs exacerbated cutaneous disease), because he never took NSAIDs during the last 25 years. Nonetheless, the fact that he was able to tolerate a whole normal dose of aspirin after his severe chronic urticaria went into remission might suggest this possibility. However, the lack of a positive aspirin challenge prior to omalizumab treatment represents an important limitation, as a natural resolution of NSAID hypersensitivity, although unlikely, cannot be excluded. Altogether, this case suggests that it might be worth to challenge with aspirin or other strong COX-1 inhibitors NECD patients showing a complete response to omalizumab. In view of their frequent autoreactivity (12) it would be interesting to investigate the effects of omalizumab in NIUA patients as well.

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Patch testing in a case of eslicarbazepine and carbamazepine induced cutaneous reaction

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Keywords
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Summary
Anticonvulsants are among the drugs most commonly involved in cutaneous adverse drug reactions (CADRs). Eslicarbazepine is a new anti-epileptic drug, chemically related to carbamazepine but with a more favorable safety profile. We report the clinical case of a woman who developed a skin rash on day 10 of eslicarbazepine with further exacerbation with eosinophilia on day 2 of carbamazepine. Epicutaneous tests were positive with eslicarbazepine.

Introduction

Eslicarbazepine is an oral anticonvulsant indicated for the adjunctive treatment of partial seizures, with or without secondary generalization, in the adult population. Eslicarbazepine acetate is a prodrug for eslicarbazepine (S-licarbazepine), which is the active metabolite of oxcarbazepine (1,2). It is contraindicated in patients with a history of hypersensitivity to other carboxamide derivatives, such as oxcarbazepine and carbamazepine, since it shares a similar chemical structure (3). Eslicarbazepine shows a better safety profile than carbamazepine (2,4); in clinical trials in adult epileptic patients, rash was reported in 1-3% of the patients taking eslicarbazepine acetate, while carbamazepine is known to cause cutaneous adverse drug reactions (cADRs) in up to 17% of patients (2,5,6).
The occurrence of cADRs is a significant problem, because it often leads to discontinuation of treatment and is usually unpredictable. Nevertheless, recent studies have revealed that human leukocyte antigen (HLA) genotypes could be possibly linked to a predisposition to cADRs induced by drugs, including carbamazepine (5,7). Since the approval of eslicarbazepine, in 2009, there have been scarce reports of hypersensitivity reactions and, currently, there are neither established risk factors for adverse reaction with eslicarbazepine acetate, nor a known correlation with the HLA genotype, as there is for carbamazepine (4,7).
We report a case of a patient with adverse skin reactions to eslicarbazepine and carbamazepine, whose skin patch tests were positive for the former compound.

Case Report

A 54-year-old woman, Caucasian, developed a slightly pruritic maculo-papular generalized skin eruption without fever or systemic symptoms on the 10th day of eslicarbazepine acetate and

Figure 1

gabapentine, prescribed for a trigeminal neuropathic pain. The patient was initially medicated with 25 mg of hidroxizine per day, and was told to maintain the previous medication. There was some improvement, but the skin rash persisted. Eleven days after the beginning of the skin eruption she was observed by her neurologist, who stopped gabapentine and eslicarbazepine and switched her to pregabalin.

After two days, due to worsening of the pain on her new therapeutic regime, the patient went to the emergency room where she was advised to stop pregabalin and started treatment with carbamazepine, metamizol and diazepam. Two days later she recurred again to the emergency because of marked worsening of the skin rash. She had a disseminated erithemato-violaceous maculo-papular eruption, along with mild edema of the face and extremities, without arthralgia or fever. Her blood tests revealed eosinophilia (14.12%; 0.8 x 10 9/L); the serum levels of creatinine, blood urea nitrogen and hepatic parameters were within normal ranges. The patient started immediately a prednisolone course, withdrawing all previous medication, and was referred to our Immunoallergology outpatient clinic. There was a progressive regression of the skin lesions, with a complete recovery within around six weeks and normalization of the eosinophil count (0.140 x 109/L, 2.5%) in blood tests performed five months later. She meanwhile started treatment with amitriptyline, which was well tolerated. The previous use of antiepileptic drugs was denied.

One month after full recovery we performed patch tests with eslicarbazepine, gabapentin, carbamazepine and pregabalin. We used commercial tablets of 800 mg eslicarbazepine, 100 mg gabapentin, 200 mg carbamazepine and 25 mg pregabalin, which were finely grounded and incorporated at 20% in white petrolatum. The tests were positive only for eslicarbazepine, both at day 2 and day 4 (**figure 1**). Six controls were patch-tested with eslicarbazepine, in the same concentration, with negative result.

Discussion

This patient suffered from a generalized maculopapular eruption with mild eosinophilia but without systemic symptoms or evidence of organic lesion, thus not fulfilling the criteria for DRESS (8). However, antiepileptic drugs, in particular carbamazepine, are among the most common causes of severe cutaneous drug reactions (3,5,8). The prompt interruption of the suspected drugs might have protected the patient from a more severe reaction (3,5,9).

Given the clinical history, with the first symptoms appearing 10 days after starting eslicarbazepine and gabapentine followed by exacerbation 2 days after starting carbamazepine, which is chemically related to eslicarbazepine, we assumed eslicarbazepine and carbamazepine as the most probable culprit drugs. The positive patch test with eslicarbazepine strengthened our diagnosis. Avoidance of these and related drugs, like oxcarbazepine, was advised.

Carbamazepine is among the drugs most often involved in cutaneous adverse reactions, and there are many reports about the usefulness of patch test for the diagnosis (5,9,10). Conversely, eslicarbazepine is rarely implicated in hypersensitivity reactions (2,4) and we found no published information concerning the use of epicutaneous testing or the determination of non irritant concentrations with this drug.

Based on the published guidelines and because we used the commercial form of the implicated drugs, we decided to test with a concentration of 20% in petrolatum (11,12). The negative result on six healthy controls allowed us to exclude an irritant reaction.

Despite the negative epicutaneous test with carbamazepine, we still consider the patient might be sensitized to this drug. There are reports of negative patch tests with carbamazepine, possibly because in some cases the reaction could be triggered by a metabolite and not by the parent compound (13,14). Moreover, drug hypersensitivity reactions tend to occur earlier and be more severe on reexposure, which was evident in our patient after she started carbamazepine. Alternatively, she could have suffered a flare-up reaction and not a cross-reactive hypersensitivity reaction to carbamazepine (15).

In conclusion, we consider this case relevant because there is a scarcity of reports on hypersensitivity reactions to eslicarbazepine. Epicutaneous tests with this drug seem to be useful for the identification of the culprit drug on cADRs. The concentration of 20% on petrolatum (with grounded tablets) elicited no irritant reactions.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Freshly squeezed: anaphylaxis caused by drone larvae juice

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Key words

venom; honey; royal jelly; propolis; beekeeping

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Summary

Drone larvae are mostly considered a by-product of beekeeping, but have recently been advocated as a high-protein source of food. There are as yet no data concerning their allergenic potential.

We report on a 29-year old bee keeper who experienced an anaphylactic reaction following the consumption of a freshly prepared beverage from raw drone larvae. Larvae-specific sensitization was confirmed by prick-to-prick and basophil activation testing. Bee stings and classical bee products including honey and royal jelly were tolerated.

This is the hitherto first report on IgE-mediated allergy to drone larvae. We suggest that a certain awareness towards the allergenicity of bee larvae is required.

Introduction

Drone larvae are commonly considered a by-product of beekeeping to be removed from the colonies for the management of Varroa mites (1). Other than honey, royal jelly, and propolis, larvae are not normally consumed in Western countries, though they have been advocated as a high-protein source of food (2). Strengthening and invigorating properties have been attributed to drone brood preparations, also referred to as "apilarnil", a homogenized mixture of male larvae, royal jelly, and residual brood combs.

Case presentation

A 29-year-old beekeeper prepared a raw drone brood juice by putting entire combs in a conventional juicer and took a small sip. He immediately experienced an itching sensation in his mouth and ears which spontaneously subsided. Some 60 minutes later, he developed a systemic anaphylactic reaction including flush and angioedema, cough, respiratory distress, nausea, vomiting, and tachycardia. He received intravenous emergency treatment with antihistamines and steroids, and was admitted to hospital for further observation. He had a history of mild allergic rhinitis and asthma, but never had asthma exacerbations while beekeeping. During the past two years, he had had about 20 bee stings and occasionally suffered from large local reactions, but never experienced sting-induced anaphylaxis. He was eating honey on a daily basis and had occasionally consumed royal jelly and propolis without symptoms. This was his first deliberate consumption of larvae. Total and bee/*Vespula* venom-specific IgE were measured using the ImmunoCAP method (Thermo Fisher Scientific, Freiburg,

Germany). Basophil activation testing was done with honey bee venom (1.0 - 0.001 µg/ml) and the supernatant of the culprit drone brood juice after centrifugation (pure and aqueous dilutions ranging from 1:5 to 1:100,000). Activated basophils (i.e. CD63 and IgE double positive cells) were measured by flow cytometry. Intradermal tests with bee/*Vespula* venom and prick-to-prick tests with a variety of bee products (drone brood juice, raw and cooked larvae, royal jelly, honey, beeswax, and propolis) were performed according to international standards, with reading after 15 minutes. Our patient was serologically sensitized to honey bee venom (total IgE 313 kU/L, bee venom-specific IgE 2.99 kU/L, IgE to rApi m1 0.99 kU/L) and had a positive intradermal test response to bee venom at 0.1 µg/L. Honey bee venom basophil activation tests, however, were negative. Prick-to-prick-tests with drone brood juice and raw larvae were clearly positive with wheals sized 10 and 6 mm, respectively (histamine positive control 5 mm). A smaller wheal (3 mm) was obtained when using a cooked larva (microwave, 45 seconds, 600 W). Basophil activation tests using centrifuged drone brood juice were positive with a maximum activation rate of 89.1% at a dilution of 1:100 (negative control 1.75%, positive control 50.5%). Prick-to-prick tests with all other bee products were negative, and oral provocation with a teaspoon of royal jelly was tolerated without symptoms. The patient was advised to avoid all preparations openly containing bee larvae and to exercise extreme caution when consuming other bee products potentially contaminated with larvae protein.

Discussion

To our knowledge, this is the hitherto first report on IgE-mediated allergy to bee larvae. Continued tolerance of honey and royal jelly and negative skin test reactivity to propolis and beeswax were suggestive of a larvae-specific sensitization. Mode and route of sensitization are open to speculation, but several aspects are worth discussion. 1) Honey bee venom allergy as the primary source of sensitization and subsequent cross-reactivity to larvae proteins was considered unlikely as the patient had never experienced anaphylactic sting reactions, and basophil activation tests with incremental doses of bee venom were invariably negative despite serological and skin test reactivity to honey bee venom. 2) Though the patient had never before deliberately consumed larvae, oral sensitization was considered an option as he might have accidently ingested residuals of larvae when harvesting royal jelly from the brood coombs. 3) In view of the patient's atopic condition, inhalation of dust particles containing larvae and/or bee body components while beekeeping was likewise assumed to be a plausible source of sensitization. Interestingly, he described mucosal itching resembling oral allergy syndrome as the first symptom of the allergic reaction, and prick tests with cooked larvae were suggestive of a certain heat-lability of the causative allergen.

The above mentioned considerations are in accordance with reports on IgE-mediated allergy to other bee-products identifying beekeeping, atopy and respiratory diseases as risk factors, whereas clinically relevant cross-reactivity to honey bee venom seems to be an exception (3). Royal jelly is a glandular secretion from worker bees used to feed all kinds of larvae and the adult queen. Royal jelly-induced anaphylaxis has been described on several occasions and may take a severe or even fatal course (4-6). Due to local nutritional habits, it is considered to be more prevalent in Asia (7). Of note, one of the above mentioned case reports does not specify the exact composition of the culprit royal jelly preparation referred to as "beverage containing crude royal jelly" (4), therefore giving rise to the question whether contaminating larvae might have been the actual elicitor of anaphylaxis. Major royal jelly proteins 8 and 9 have been identified as glycosylated components of bee venom (Api m11), whereas their clinical relevance for sting-induced anaphylactic reactions remains uncertain (3,8). Furthermore, royal jelly has been recognized as a cause of occupational allergic respiratory disease (9). Honey is a sporadic elicitor of immediate-type allergic reactions which may range from oral allergy syndrome to severe anaphylaxis, and have been attributed to residual bee secretions, body parts, and/or pollen proteins (10). Propolis, a resinous mixture used as a sealant, represents a frequently recognized contact allergen rather than an elicitor of anaphylaxis (3).

Conclusion

Larvae ought to be added to the list of potentially allergenic bee products. Our observations are unlikely to alter current dietary habits in Western countries as most people would abstain from the consumption of larvae due to disgust rather than for the fear of allergy. They might, however, be of global interest as eating insects is a common practice in many developing countries (2).

Conflict of interest

The authors declare that they have no conflict of interest.

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Fatal asthma after omalizumab and controller therapy discontinuation

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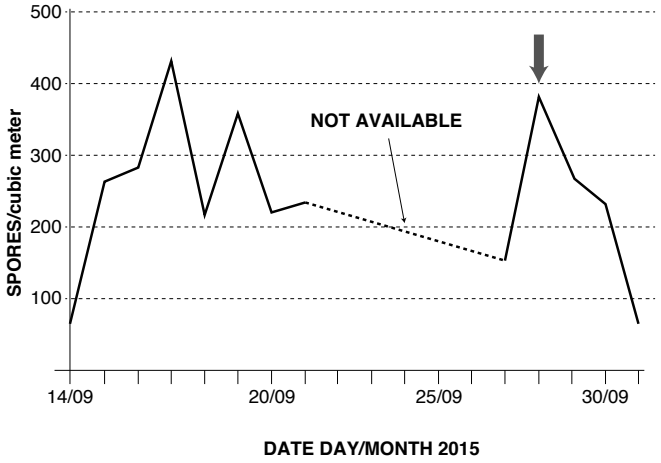
To the Editor

Severe asthma (and near-fatal asthma) account for the majority of socio-economic and healthcare expenses. Severe asthma is characterized by the absence of control despite the maximal standard therapy (often requiring courses of systemic corticosteroids) (1), whereas near-fatal asthma implies respiratory failure with/without mechanical ventilation (2). During the last decade, the more detailed knowledge of the pathogenic mechanisms and the availability of biological agents (i.e. monoclonal antibodies) improved the therapeutic approaches (3). For historical and scientific reasons, the anti-IgE therapy (omalizumab) was the first available biological treatment used in severe allergic asthma. The clinical efficacy and safety of omalizumab is well documented in trials and real life (4), whereas it is not fully elucidated yet whether the treatment should be continued life-long or if it could be discontinued after some years (5).
We describe a clinical case of fatal asthma occurred after the discontinuation of omalizumab in a patient with severe asthma.

The patient was a 28 year-old female, never smoker, referred to our services in 2007. She suffered from severe allergic asthma (> 2 exacerbations/year), with ascertained sensitization (Immunocap® Thermo Fisher Scientific, Uppsala, Sweden) to: mite (24.10 kU/L), cat dander (7.99 kU/L), olive (20.10 kU/L), *Alternaria* (88 kU/L) and grass (> 100 kU/L). The total IgE concentration was 323 kU/L (Phadia Diagnostics, Upssala, Sweden), exhaled nitric oxide was 132 ppb, and the asthma control test score was 12 at baseline. The clinical history documented some exacerbations during thunderstorms, emergency department admissions, and hospitalizations due to asthma attacks. After the baseline assessment, a treatment with omalizumab, in addition to standard treatment (6), was instituted (375 mcg subcutaneously every 4 weeks). A progressive improvement was observed from both the clinical and functional viewpoint. In particular, FEV1 increased from 72% to 94% of predicted value within few weeks, and then remained stable. The Asthma Control Test score never fell below 21 points. Noticeably, after starting omalizumab there

Figure 1 *- Alternaria spores count (Sep 14 - Oct 1 2015) in the area (Verona, Garda Lake) where the patient resided (Public bulletin from the Dipartimento Regionale per la Sicurezza del Territorio, ARPAV, Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto). Y axis, spores/m³; X axis, day and month. The blue arrow marks the day of the fatal event.*



were no more exacerbations, hospitalizations or emergency visits, and the controller therapy could be gradually reduced. The treatment was carried on from March 2007 to December 2012, for a total of 67 injections. According to the favorable and stable outcome, to the available literature (6) and to the willingness of the patient (also related to logistic aspects), the treatment was discontinued in December 2012. After omalizumab discontinuation, regular control visits were carried out until January 2015, then the patient was lost to follow-up. Until 2015 she reported only mild symptoms of asthma (< 2/month), well controlled with inhaled albuterol. On September 28 2015 the patient was admitted to emergency room for a severe asthma attack with respiratory failure, needing invasive mechanical ventilation. The patient died, despite the heavy medical therapy (high dose intravenous steroids, adrenaline, magnesium sulphate, theophylline) and the strenuous resuscitation manoeuvers. The autopsy confirmed the presence of severe bronchial obstruction, with eosinophil and neutrophil infiltration. Subsequently, we ascertained, on a historical basis, that the patient had recently increased the use of inhaled albuterol, and that she was not taking controller drugs for asthma. In addition, looking at the aerobiological data we noticed that the level of *Alternaria* sporae was remarkably high, in the absence of thunderstorm-like conditions (**figure 1**), and that the patient was strongly sensitized to this allergen.

The above described case evidences that: i) Asthma mortality still represents a critical issue in the management of the disease, particularly in youngsters) (7); ii) the role of "minor" but hazardous allergens as *Alternaria* can be underestimated in everyday clinical practice (8), thus the importance of the availability of aeroallergen count has to be underlined; iii) it would be advisable not to discontinue omalizumab in patients with severe/near-fatal asthma who had previously achieved a satisfactory control. This latter fact remains controversial and matter of debate. There are studies showing that a relevant part of patients achieve a stable improvement after omalizumab discontinuation (9), and other studies would suggest that a prolonged (possibly life-long) therapy would produce more benefits (5). Indeed, this aspect is strictly dependent on the initial asthma diagnosis, on the type of allergic sensitization, and probably on the presence of previous near-fatal asthma, that is the most relevant risk factor for subsequent episodes (10). Our experience, as described above, would suggest caution in stopping omalizumab treatment in patients with severe asthma and near-fatal asthma sensitized to perennial allergens (i.e. *Alternaria*), which are recognized as particularly harmful. Finally, we have to consider that probably the patient reduced or stopped the use of controller drugs, and this is difficult to assess, whereas the administration of a subcutaneous drug (omalizumab) needing medical supervision is easier to certify.

Conflict of interest and funding

The authors declare that they have no conflict of interest.

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BILASTINA

Ayrinal

RIASSUNTO DELLE CARATTERISTICHE DEL PRODOTTO

1. DENOMINAZIONE DEL MEDICINALE. AYRINAL 20 mg compresse. **2. COMPOSIZIONE QUALITATIVA E QUANTITATIVA.** Ogni compressa contiene 20 mg di bilastina. Per l'elenco completo degli eccipienti, vedere paragrafo 6.1. **3. FORMA FARMACEUTICA.** Compressa. Compresse bianche, ovali, biconvesse con linea di incisione (lunghezza 10 mm, larghezza 5 mm). La linea di incisione sulla compressa serve solo per agevolarne la rottura al fine di ingerire la compressa più facilmente e non per dividerla in dosi uguali. **4. INFORMAZIONI CLINICHE. 4.1 Indicazioni terapeutiche.** Trattamento sintomatico della rinocongiuntivite allergica (stagionale e perenne) e dell'orticaria. AYRINAL è indicato negli adulti e negli adolescenti (12 anni di età ed oltre). **4.2 Posologia e modo di somministrazione. Posologia:** *Adulti e adolescenti* (12 anni di età ed oltre): 20 mg di bilastina (1 compressa) una volta al giorno per alleviare i sintomi della rinocongiuntivite allergica (SAR e PAR) e dell'orticaria. La compressa deve essere assunta un'ora prima o due ore dopo l'assunzione di cibo o succhi di frutta (vedere paragrafo 4.5). *Popolazioni speciali:* Anziani: Non sono necessari aggiustamenti del dosaggio nei pazienti anziani (vedere paragrafi 5.1 e 5.2). Insufficienza renale: Non sono necessari aggiustamenti del dosaggio nei pazienti con compromissione renale. (vedere paragrafo 5.2). Insufficienza epatica: Non esiste esperienza clinica in pazienti con compromissione epatica. Dato che la bilastina non viene metabolizzata e la clearance renale è la principale via di eliminazione, non si prevede che la compromissione epatica aumenti l'esposizione sistemica oltre il margine di sicurezza. Pertanto, non è necessario alcun aggiustamento del dosaggio nei pazienti con compromissione epatica (vedere paragrafo 5.2). Popolazione pediatrica: L'uso specifico di bilastina nei bambini di età compresa tra 0 e 2 anni nelle indicazioni rinocongiuntivite allergica ed orticaria non è documentato. La sicurezza e l'efficacia di bilastina nei bambini di età inferiore ai 12 anni non sono state ancora stabilite. Durata del trattamento: Per la rinite allergica il trattamento deve essere limitato al periodo di esposizione agli allergeni. Per la rinite allergica stagionale il trattamento può essere interrotto dopo la scomparsa dei sintomi e ripreso alla loro ricomparsa. Nella rinite allergica perenne può essere proposto ai pazienti un trattamento continuato durante il periodo di esposizione agli allergeni. Nell'orticaria la durata del trattamento dipende dal tipo, dalla durata e dal decorso dei disturbi. Modo di somministrazione: Uso orale: La compressa deve essere deglutita con acqua. Si raccomanda di assumere la dose giornaliera in un'unica somministrazione. **4.3 Controindicazioni.** Ipersensibilità al principio attivo o ad uno qualsiasi degli eccipienti elencati al paragrafo 6.1. **4.4 Avvertenze speciali e precauzioni d'impiego. Popolazione pediatrica:** La sicurezza e l'efficacia della bilastina nei bambini al di sotto dei 12 anni di età non sono state stabilite. Nei pazienti con compromissione renale da moderata a grave la co-somministrazione della bilastina con inibitori della P-glicoproteina, quali ad esempio chetoconazolo, eritromicina, ciclosporina, ritonavir o diltiazem, può aumentare i livelli plasmatici della bilastina e pertanto aumentare il rischio di reazioni avverse. Pertanto, la co-somministrazione della bilastina ed inibitori della P-glicoproteina deve essere evitata in pazienti con compromissione renale da moderata a grave. **4.5 Interazioni con altri medicinali ed altre forme di interazione.** Interazione con il cibo: il cibo riduce significativamente la biodisponibilità orale della bilastina del 30%. Interazione con il succo di pompelmo: l'assunzione concomitante della bilastina 20 mg con il succo di pompelmo diminuisce la biodisponibilità della bilastina del 30%. Questo effetto può verificarsi anche con altri succhi di frutta. Il grado di diminuzione della biodisponibilità può variare a seconda dei diversi produttori e dei frutti. Il meccanismo di questa interazione è l'inibizione dell'OATP1A2, un trasportatore di uptake per il quale la bilastina è un substrato (vedere paragrafo 5.2). I medicinali che sono substrati o inibitori dell'OATP1A2, come ritonavir o rifampicina, possono analogamente avere il potenziale di diminuire la concentrazione plasmatica della bilastina. Interazione con chetoconazolo o eritromicina: l'assunzione concomitante della bilastina e chetoconazolo o eritromicina ha aumentato l'AUC della bilastina di 2 volte e la C_{max} di 2-3 volte. Questi cambiamenti possono essere spiegati dall'interazione con le proteine di trasporto intestinale, in quanto la bilastina è un substrato per P-gp e non viene metabolizzata (vedere paragrafo 5.2). Questi cambiamenti non sembrano avere effetti sul profilo di sicurezza della bilastina e chetoconazolo o eritromicina, rispettivamente. Analogamente altri medicinali che sono substrati o inibitori di P-gp, come la ciclosporina, possono potenzialmente aumentare la concentrazione plasmatica della bilastina. Interazione con diltiazem: l'assunzione concomitante della bilastina 20 mg e diltiazem 60 mg ha aumentato la C_{max} della bilastina del 50%. Questo effetto può essere spiegato dall'interazione con le proteine di trasporto intestinale (vedere paragrafo 5.2) e non sembra avere effetti sul profilo di sicurezza della bilastina. Interazione con alcool: la performance psicomotoria dopo l'assunzione concomitante di alcool e della bilastina 20 mg è stata simile a quella osservata dopo l'assunzione di alcool e placebo. Interazione con lorazepam: l'assunzione concomitante della bilastina 20 mg e lorazepam 3 mg per 8 giorni non ha potenziato gli effetti sedativi sul SNC del lorazepam. Popolazione pediatrica: Sono stati effettuati studi di interazione solo negli adulti. Il grado di interazione con altri medicinali ed altre forme di interazione dovrebbero essere simili nella popolazione pediatrica di età compresa tra i 12 e i 17 anni di età. **4.6 Fertilità, gravidanza e allattamento.** Gravidanza: i dati relativi all'uso della bilastina in donne in gravidanza non esistono o sono in numero limitato. Studi condotti sugli animali non indicano la presenza di effetti negativi diretti o indiretti riguardanti la tossicità riproduttiva, il parto o lo sviluppo postnatale (vedere paragrafo 5.3). A scopo precauzionale, è preferibile evitare l'uso di AYRINAL durante la gravidanza. Allattamento: L'escrezione della bilastina nel latte non è stata studiata nell'uomo. I dati farmacocinetici disponibili sugli animali hanno evidenziato escrezione della bilastina nel latte (vedere paragrafo 5.3). La decisione in merito ad interrompere/astenersi dalla terapia con AYRINAL deve tenere in considerazione il beneficio dell'allattamento per il bambino e il beneficio della terapia con la bilastina per la madre. Fertilità: non esistono dati clinici oppure sono in numero limitato. Uno studio condotto nei ratti non ha indicato alcun effetto negativo sulla fertilità (vedere paragrafo 5.3). **4.7 Effetti sulla capacità di guidare veicoli e sull'uso di macchinari.** Uno studio eseguito per valutare gli effetti della bilastina sulla capacità di guidare ha dimostrato che il trattamento con 20 mg non ha influenzato la capacità di guida. Tuttavia, i pazienti devono essere avvertiti che molto raramente in alcune persone si è manifestata sonnolenza, che può influenzare la capacità di guidare veicoli o usare macchinari. **4.8 Effetti indesiderati. Sintesi del profilo di sicurezza:** L'incidenza di eventi avversi in pazienti affetti da rinocongiuntivite allergica o da orticaria idiopatica cronica trattati con 20 mg di bilastina nei trial clinici è stato paragonabile all'incidenzain pazienti trattati con placebo (12,7% rispetto a 12,8%). Durante lo sviluppo clinico, sono stati condotti studi di fase II e III che hanno incluso 2525 pazienti trattati con diversi dosaggi di bilastina, di cui 1697 sono stati trattati con bilastina 20 mg. In questi studi 1362 pazienti hanno ricevuto placebo. Le reazioni avverse più comunemente segnalate dai pazienti che hanno ricevuto 20 mg di bilastina per l'indicazione rinocongiuntivite allergica o orticaria idiopatica cronica sono state mal di testa, sonnolenza, capogiri e affaticamento. Questi eventi avversi si sono verificati con una frequenza paragonabile nei pazienti trattati con placebo. ***Tabella riassuntiva delle reazioni avverse:*** Nella tabella che segue sono riportate le reazioni avverse possibilmente correlate alla bilastina e segnalate in oltre lo 0,1% dei pazienti trattati con 20 mg di bilastina nel corso dello sviluppo clinico (N = 1697). Le frequenze sono assegnate come segue: Molto comune (≥1/10); Comune (da ≥1/100 a <1/10); Non comune (da ≥1/1.000 a <1/100); Raro (da ≥1/10.000 a <1/1.000); Molto raro (<1/10.000); Non nota (la frequenza non può essere definita sulla base dei dati disponibili). Le reazioni rare, molto rare e con frequenza non nota non sono state incluse nella tabella.

Classificazione per Sistemi ed Organi		**Bilastina 20 mg N=1697**	**Bilastina Tutte le dosi N=2525**
Frequenza	**Reazione avversa**		
Infezioni e infestazioni			
Non comune	***Herpes orale***	2 (0,12%)	2 (0,08%)
Disturbi del metabolismo e della nutrizione			
Non comune	***Aumento dell'appetito***	10 (0,59%)	11 (0,44%)
Disturbi psichiatrici			
Non comune	***Ansia***	6 (0,35%)	8 (0,32%)
	Insonnia	2 (0,12%)	4 (0,16%)
Disturbi del sistema nervoso			
Comune	***Sonnolenza***	52 (3,06%)	82 (3,25%)
	Cefalea	68 (4,01%)	90 (3,56%)
Non comune	***Capogiri***	14 (0,83%)	23 (0,91%)
Disturbi dell'orecchio e del labirinto			

Classificazione per Sistemi ed Organi		Bilastina 20 mg N=1697	Bilastina Tutte le dosi N=2525
Frequenza	**Reazione avversa**		
Non comune	***Tinnito***	2 (0,12%)	2 (0,08%)
	Vertigini	3 (0,18%)	3 (0,12%)
Patologie cardiache			
Non comune	***Blocco di branca destra***	4 (0,24%)	5 (0,20%)
	Aritmia sinusale	5 (0,30%)	5 (0,20%)
	Prolungamento del tratto QT all'elettrocardiogramma	9 (0,53%)	10 (0,40%)
	Altre anomalie all'ECG	7 (0,41%)	11 (0,44%)
Patologie respiratorie, toraciche e mediastiniche			
Non Comune	***Dispnea***	2 (0,12%)	2 (0,08%)
	Fastidio nasale	2 (0,12%)	2 (0,08%)
	Secchezza del naso	3 (0,18%)	6 (0,24%)
Patologie gastrointestinali			
Non comuni	***Dolore all'addome superiore***	11 (0,65%)	14 (0,55%)
	Dolore addominale	5 (0,30%)	5 (0,20%)
	Nausea	7 (0,41%)	10 (0,40%)
	Fastidio gastrico	3 (0,18%)	4 (0,16%)
	Diarrea	4 (0,24%)	6 (0,24%)
	Bocca secca	2 (0,12%)	6 (0,24%)
	Dispepsia	2 (0,12%)	4 (0,16%)
	Gastrite	4 (0,24%)	4 (0,16%)
Patologie della cute e del tessuto sottocutaneo			
Non comune	***Prurito***	2 (0,12%)	4 (0,16%)
Disturbi generali e condizioni relative alla sede di somministrazione			
Non comune	***Affaticamento***	14 (0,83%)	19 (0,75%)
	Sete	3 (0,18%)	4 (0,16%)
	Miglioramento della condizione pre-esistente	2 (0,12%)	2 (0,08%)
	Piressia	2 (0,12%)	3 (0,12%)
	Astenia	3 (0,18%)	4 (0,16%)
Esami disgnostici			
Non comune	***Aumento della gamma-glutamiltransferasi***	7 (0,41%)	8 (0,32%)
	Aumento dell'alanina amino transferasi	5 (0,30%)	5 (0,20%)
	Aumento dell'aspartato aminotransferasi	3 (0,18%)	3 (0,12%)
	Aumento della creatinina nel sangue	2 (0,12%)	2 (0,08%)
	Aumento dei trigliceridi nel sangue	2 (0,12%)	2 (0,08%)
	Aumento del peso corporeo	8 (0,47%)	12 (0,48%)

Frequenza non nota (non può essere definita sulla base dei dati disponibili): palpitazioni, tachicardia e reazioni di ipersensibilità (quali anafilassi, angioedema, dispnea, eruzione cutanea, edema localizzato/gonfiore locale ed eritema) sono state osservate nel periodo post-marketing. Descrizione di alcune reazioni avverse: Le reazioni avverse segnalate con maggior frequenza sono state due comuni (sonnolenza e cefalea) e due non comuni (capogiri e affaticamento). Le loro frequenze in pazienti trattati con bilastina rispetto ai pazienti trattati con placebo sono state 3.06% vs. 2.86% per la sonnolenza; 4.01% vs. 3.38% per la cefalea; 0.83% vs. 0.59% per i capogiri; 0.83% vs. 1.32% per l'affaticamento. Quasi tutte le reazioni avverse, incluse nella tabella sopra riportata, sono state osservate con un'incidenza simile sia in pazienti trattati con 20 mg di bilastina che in quelli trattati con placebo. Le informazioni raccolte nel corso della vigilanza post-marketing hanno confermato il profilo di sicurezza osservato durante lo sviluppo clinico . Popolazione pediatrica: La frequenza, la tipologia e la severità delle reazioni avverse negli adolescenti (di età compresa tra 12 e 17 anni) durante lo sviluppo clinico, sono state le stesse osservate negli adulti. Le informazioni raccolte in questa popolazione (adolescenti) durante la vigilanza post-marketing hanno confermato i risultati degli studi clinici. Segnalazione delle reazioni avverse sospette: La segnalazione delle reazioni avverse sospette che si verificano dopo l'autorizzazione del medicinale è importante, in quanto permette un monitoraggio continuo del rapporto beneficio/rischio del medicinale. Agli operatori sanitari è richiesto di segnalare qualsiasi reazione avversa sospetta tramite il sistema nazionale di segnalazione all'indirizzo: http://www.agenziafarmaco.gov.it/content/come-segnalare-una-sospetta-reazione-avversa. **4.9 Sovradosaggio.** Le informazioni inerenti il sovradosaggio acuto di bilastina derivano dalle esperienze raccolte in trial clinici condotti durante lo sviluppo e la vigilanza post-marketing. Nel corso degli studi clinici, dopo la somministrazione di bilastina a dosi superiori di 10 o 11 volte la dose terapeutica (220 mg (dose singola); o 200 mg/die per 7 giorni) a volontari sani, la frequenza degli eventi avversi occorsi durante il trattamento è stata di due volte superiore rispetto al placebo. Le reazioni avverse segnalate con maggior frequenza sono state capogiri, cefalea e nausea. Non sono stati segnalati eventi avversi gravi e nessun prolungamento significativo nell'intervallo QTc. Le informazioni raccolte nel corso della vigilanza post-marketing sono in linea con quanto riportato negli studi clinici. Una valutazione critica dell'effetto di dosi multiple di bilastina (100 mg x 4 giorni) sulla ripolarizzazione ventricolare mediante un "approfondito studio incrociato sul QT/QTc" che ha coinvolto 30 volontari sani, non ha evidenziato un prolungamento significativo del QTc. In caso di sovradosaggio si raccomanda un trattamento sintomatico e di supporto. Non esiste alcun antidoto noto alla bilastina. **5. PROPRIETÀ FARMACOLOGICHE. 5.1 Proprietà farmacodinamiche.** Categoria farmacoterapeutica: antistaminici per uso sistemico, altri antistaminici per uso sistemico. Codice ATC R06AX29. La bilastina è un'antagonista istaminergico non sedativo, ad azione prolungata con selettiva affinità antagonista per il recettore H_1 periferico e nessuna affinità per i recettori muscarinici. La bilastina ha inibito reazioni cutanee eritemato-pomfoidi indotte dall'istamina per 24 ore in seguito a somministrazioni di dosi singole. Nei trial clinici eseguiti in pazienti adulti ed adolescenti con rinocongiuntivite allergica (stagionale e perenne), la bilastina 20 mg, somministrata una volta al giorno per 14-28 giorni, è stata efficace nell'alleviare i sintomi quali starnuti, fastidio nasale, prurito nasale, congestione nasale, prurito agli occhi, lacrimazione e rossore oculare. La bilastina ha mantenuto efficacemente sotto controllo i sintomi per 24 ore. In due trial clinici condotti in pazienti con orticaria idiopatica cronica, la bilastina 20 mg, somministrata una volta al giorno per 28 giorni è stata efficace nell'alleviare l'intensità del prurito ed il numero e le dimensioni dei pomfi, oltre ai disturbi provocati dall'orticaria. Nei pazienti sono migliorate le condizioni del sonno e la qualità della vita. Nei trial clinici condotti con la bilastina non è stato osservato un prolungamento clinicamente rilevante dell'intervallo QTc o alcun altro effetto cardiovascolare, anche a dosi di 200 mg al giorno (10 volte la dose clinica) per 7 giorni in 9 soggetti, oppure anche quando co-somministrata con inibitori P-gp, quali chetoconazolo (24 soggetti) ed eritromicina (24 soggetti). Inoltre è stato eseguito un studio approfondito sul QT su 30 volontari. Nei trial clinici controllati alla dose raccomandata di 20 mg una volta al giorno, il profilo di sicurezza per il SNC della bilastina è stato simile al placebo e l'incidenza della sonnolenza non è stata statisticamente diversa dal placebo. La bilastina a dosi fino a 40 mg ogni giorno non ha influenzato la performance psicomotoria nei trial clinici e non ha influenzato la capacità di guida in un test di guida standard. Nei pazienti anziani (≥ 65 anni) inclusi in studi di fase II e III non sono state evidenziate differenze nell'efficacia o nella sicurezza rispetto ai pazienti più giovani. Uno studio post-autorizzativo su 146 pazienti anziani, non ha mostrato differenze sul profilo di sicurezza rispetto alla popolazione adulta. Popolazione pediatrica: Gli adolescenti (di età compresa tra 12 e 17 anni) sono stati inclusi nello sviluppo clinico. Nel corso degli studi clinici la bilastina è stata somministrata a 128 adolescenti (81 in studi in doppio cieco sulla rinocongiuntivite allergica). Un ulteriore gruppo di 116 adolescenti è stato randomizzato per la somministrazione di comparatori attivi o placebo. Non è stata osservata alcuna differenza in efficacia e sicurezza tra adulti e adolescenti. L'agenzia Europea dei Medicinali ha rinviato l'obbligo di presentare i risultati degli studi con AYRINAL in uno o più sottogruppi della popolazione pediatrica per il trattamento della rinocongiuntivite allergica e per il trattamento dell'orticaria (vedere paragrafo 4.2 per informazioni sull'uso pediatrico). **5.2 Proprietà farmacocinetiche.** Assorbimento: La bilastina viene rapidamente assorbita dopo la somministrazione orale raggiungendo la massima concentrazione nel plasma in circa 1,3 ore. Non si è osservato fenomeno di accumulo. La biodisponibilità media della bilastina dopo somministrazione orale è del 61%. Distribuzione: Studi *in vitro* e *in vivo* hanno mostrato che la bilastina è un substrato del Pgp (vedere paragrafo 4.5 "Interazione con chetoconazolo, eritromicina e diltiazem") e OATP (vedere paragrafo 4.5 "Interazione con succo di pompelmo"). La bilastina non risulta essere un substrato del trasportatore BCRP o dei trasportatori renali OCT2, OAT1

e OAT3. In base agli studi *in vitro*, non si prevede che la bilastina inibisca i seguenti trasportatori nella circolazione sistemica: P-gp, MRP2, BCRP, BSEP, OATP1B1, OATP1B3, OATP2B1, OAT1, OAT3, OCT1, OCT2 e NTCP, poiché solo una modesta inibizione è stata rilevata per P-gp, OATP2B1 e OCT1, con una IC_{50} stimata ≥ a 300 µM, molto più elevata rispetto alla C_{MAX} plasmatica clinica calcolata e per ciò queste interazioni non saranno clinicamente rilevanti. Tuttavia, sulla base di questi risultati, l'azione inibitoria della bilastina sui trasportatori presenti nella mucosa intestinale, per esempio P-gp, non può essere esclusa. Alle dosi terapeutiche la bilastina è legata per l'84-90% alle proteine del plasma. Biotrasformazione: La bilastina non ha indotto o inibito l'attività degli isoenzimi CYP450 negli studi *in vitro*. Eliminazione: In uno studio di bilanciamento di massa condotto su volontari sani, dopo la somministrazione di una singola dose di 20 mg di ^{14}C-bilastina, quasi il 95% della dose somministrata è stata recuperata nelle urine (28,3%) e nelle feci (66,5%) come bilastina immodificata, confermando quindi che la bilastina non è significativamente metabolizzata nell'uomo. L'emivita media di eliminazione calcolata in volontari sani è stata di 14,5 h. Linearità: La bilastina presenta una farmacocinetica lineare nell'intervallo di dosi studiato (da 5 a 220 mg), con bassa variabilità interindividuale. Compromissione renale: In uno studio in soggetti con compromissione renale, la media (DS) dell'$AUC_{0-\infty}$ è aumentata da 737,4 (±260,8) ngxh/ml nei soggetti senza compromissione (GFR: > 80 ml/min/1,73 m^2) a: 967,4 (±140,2) ngxh/ml nei soggetti con compromissione lieve (GFR: 50-80 ml/min/1,73 m^2), 1384,2 (±263,23) ngxh/ml nei soggetti con compromissione moderata (GFR: 30 - <50 ml/min/1,73 m^2), e 1708,5 (±699,0) ngxh/ml nei soggetti con compromissione grave (GFR: < 30 ml/min/1,73 m^2). L'emivita media (DS) della bilastina era 9,3 h (± 2,8) nei soggetti senza compromissione, 15,1 h (± 7,7) nei soggetti con compromissione lieve, 10,5 h (± 2.3) nei soggetti con compromissione moderata e 18,4 h (± 11,4) nei soggetti con compromissione grave. L'escrezione urinaria della bilastina era essenzialmente completa dopo 48-72 h in tutti i soggetti. Questi cambiamenti farmacocinetici non si prevede presentino un'influenza clinicamente rilevante sulla sicurezza della bilastina, dato che i livelli di bilastina nel plasma nei pazienti con compromissione renale rientrano ancora nell'intervallo di sicurezza della bilastina. Compromissione epatica: Non esistono dati sulla farmacocinetica per i soggetti con compromissione epatica. La bilastina non viene metabolizzata negli umani. Dato che i risultati dello studio sulla compromissione renale indicano che l'eliminazione renale è il maggior contribuente dell'eliminazione, si prevede che l'escrezione biliare sia coinvolta solo marginalmente nell'eliminazione di bilastina. Non si prevede che le alterazioni nella funzione epatica abbiamo un'influenza clinicamente rilevante sulla farmacocinetica di bilastina. Anziani: Sono disponibili solo un quantitativo limitato di dati di studi farmacocinetici in soggetti oltre i 65 anni di età. Non sono state osservate differenze statisticamente significative nella farmacocinetica della bilastina negli anziani oltre i 65 anni di età rispetto alla popolazione di adulti di età compresa tra 18 e 35 anni. Popolazione pediatrica: Non sono disponibili dati di farmacocinetica negli adolescenti (di età compresa tra 12 e 17 anni) in quanto, per questo prodotto, l'estrapolazione dei dati nell'adulto sono ritenuti appropriati. **5.3 Dati preclinici di sicurezza.** I dati non-clinici sulla bilastina non evidenziano rischi particolari per l'uomo sulla base di studi convenzionali di sicurezza farmacologica, tossicità a dosi ripetute, genotossicità e potenziale cancerogeno. Negli studi di tossicità riproduttiva gli effetti della bilastina sul feto (perdita pre-e post-impianto nei ratti ed ossificazione incompleta delle ossa craniali, dello sterno e degli arti nei conigli) sono stati osservati solo a dosi tossiche per la madre. I livelli di esposizione al NOAEL (No Observed Adverse Effect Level) sono sufficientemente in eccesso (> 30 volte) rispetto all'esposizione umana alla dose terapeutica raccomandata. In uno studio sull'allattamento, è stata riscontrata bilastina nel latte dei ratti in allattamento cui era stata somministrata una singola dose orale (20 mg/kg). Le concentrazioni di bilastina presenti nel latte equivalgono a circa la metà di quelle presenti nel plasma materno. La rilevanza di questi risultati nell'uomo non è nota. In uno studio di fertilità nei ratti, la bilastina somministrata per via orale fino a 1000 mg/kg/die non ha indotto alcun effetto sugli organi riproduttivi maschili e femminili. Gli indici di accoppiamento, fertilità e gravidanza non sono stati influenzati. Come evidenziato in uno studio di distribuzione nei ratti mediante determinazione delle concentrazioni di farmaco tramite autoradiografia, la bilastina non si accumula nel SNC. **6. INFORMAZIONI FARMACEUTICHE. 6.1 Elenco degli eccipienti.** Cellulosa microcristallina, Sodio Amido glicolato (tipo A) (derivato dalle patate), Silice colloidale anidra, Magnesio stearato. **6.2 Incompatibilità.** Non pertinente. **6.3 Periodo di validità.** 5 anni. **6.4 Precauzioni particolari per la conservazione.** Questo medicinale non richiede alcuna condizione particolare di conservazione. **6.5 Natura e contenuto del contenitore.** Il medicinale è confezionato in un blister, che consiste di due parti: laminato, composto da poliamide orientata (lato esterno del laminato), alluminio e PVC (lato interno del laminato); Foglio in allumino: Il foglio in allumino è termosaldato con una lacca termosaldante (copolimero PVC-PVAC e resine di butilmetacrilato) al laminato dopo la formatura e il riempimento con le compresse. Ciascun blister contiene 10 compresse. I blister sono confezionati in astucci di cartone. Confezioni da 10, 20, 30, 40 o 50 compresse. È possibile che non tutte le confezioni siano commercializzate. **6.6 Precauzioni particolari per lo smaltimento e la manipolazione.** Il medicinale non utilizzato ed i rifiuti derivati da tale medicinale devono essere smaltiti in conformità alla normativa locale vigente. **7. TITOLARE DELL'AUTORIZZAZIONE ALL'IMMISSIONE IN COMMERCIO.** Menarini International Operations Luxembourg S.A. 1, Avenue de la Gare, L-1611 - Lussemburgo. *Concessionario per la vendita:* Malesci Istituto Farmacobiologico S.p.A. Via Lungo l'Ema, 7 - Loc. Ponte a Ema, Bagno a Ripoli - Firenze. **8. NUMERO(I) DELL'AUTORIZZAZIONE PER L'IMMISSIONE IN COMMERCIO.** AYRINAL 20 mg compresse: 10 compresse - A.I.C. 040854010, 20 compresse - A.I.C. 040854022, 30 compresse - A.I.C. 040854034, 40 compresse - A.I.C. 040854046, 50 compresse - A.I.C. 040854059 **9. DATA DELLA PRIMA AUTORIZZAZIONE / RINNOVO DELL'AUTORIZZAZIONE.** Data di prima autorizzazione: 3 Aprile 2012. Data del rinnovo più recente: 8 settembre 2015. **10. DATA DI REVISIONE DEL TESTO.** Gennaio 2018.

CONFEZIONI	PREZZO AL PUBBLICO	CLASSE	NOTA
20 mg 20 cpr	10,80	C	-

Medicinale soggetto a prescrizione medica

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