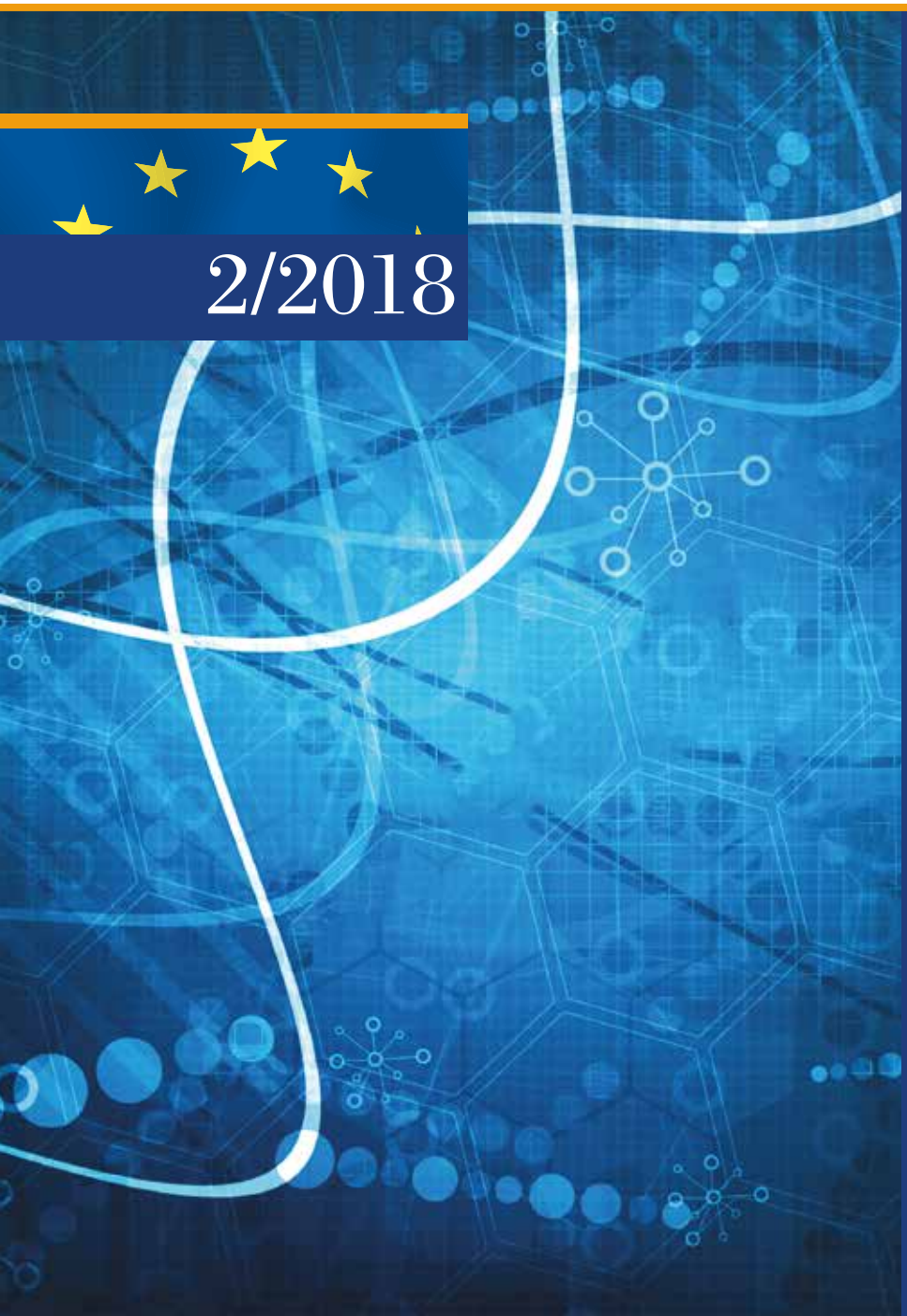
edra | ISSN 1764-1489 | VOLUME 50 N. 2/2018 – MARCH 2018

European Annals of Allergy and Clinical Immunology

THE OFFICIAL JOURNAL OF AAIITO | ASSOCIAZIONE ALLERGOLOGI IMMUNOLOGI ITALIANI TERRITORIALI E OSPEDALIERI
THE OFFICIAL JOURNAL OF SPAIC | SOCIEDADE PORTUGUESA DE ALERGOLOGIA E IMUNOLOGIA CLINICA



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"European Annals of Allergy and Clinical Immunology" registered at Tribunale di Milano - n. 336 on 22.10.2014

The contents of this Journal are indexed in PubMed, SCOPUS and Web of Science®

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Recommendations for the use of molecular diagnostics in the diagnosis of allergic diseases

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Key words

allergy; molecular diagnostics; component resolved diagnostics; guidelines; specific IgE

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Doi
10.23822/EurAnnACI.1764-1489.32

Summary

The Study Group on Allergology of the Italian Society of Clinical Pathology and Laboratory Medicine (SIPMeL) and the Associazione Italiana degli Allergologi e Immunologi Territoriali e Ospedalieri (AAIITO) developed the present recommendations on the diagnosis of allergic diseases based on the use of molecular allergenic components, whose purpose is to provide the pathologists and the clinicians with information and algorithms enabling a proper use of this second-level diagnostics. Molecular diagnostics allows definition of the exact sensitization profile of the allergic patient. The methodology followed to develop these recommendations included an initial phase of discussion between all the components to integrate the knowledge derived from scientific evidence, a revision of the recommendations made by Italian and foreign experts, and the subsequent production of this document to be disseminated to all those who deal with allergy diagnostics.

Background

- Many allergens are antigenically extremely complex.
- An extract is a mixture of proteins, only part of whom are allergens.
- Every allergic subject responds to one or more allergen based on his/her genetic background.
- A number of cross-reacting allergens exist, and are variably distributed throughout plants and animals: their structural homology is variable. A minimum 35-40% amino acid homology is needed to cause cross-reactivity.
- Both conformational and linear epitopes exist.
- There are marked differences in molecular sensitization patterns between different geographical regions.

Usefulness of component resolved diagnosis (CRD)

Molecules show a well-defined composition, can be quantified precisely, lack non-allergenic components, and can be produced in

large amounts in-vitro. CRD defines the precise allergy profile of each individual patient (1). In patients showing multi-sensitization using traditional extracts, this leads to discriminate genuine sensitizations from sensitizations caused by cross-reacting molecules (2). In respiratory allergy, this translates into the choice of the correct immunotherapy (3,4); in food allergy in the correct evaluation of the risk of severe allergic reactions based on the physical / chemical characteristics of the relevant allergens (5); in latex allergy in the correct identification of true allergic subjects needing a latex-safe environment (6); and in hymenoptera venom allergy in discriminating between honey bee and wasp allergy and within the wasp family, looking for the right venom immunotherapy (7).

Main classes of molecules

Molecules list is regularly updated into the *Official list of allergens* of the *International Union of Immunological Societies Allergen Nomenclature sub-committee* of the WHO (WHO/IUIS) (http://www.allergen.org). Although molecules may belong to more than 120 protein families, allergens responsible for most allergic reactions belong to few protein families characterized by a limited number of biologic functions (8).

Respiratory allergy

Table I (p. 54) shows the major and minor specific molecular components along with the main cross-reacting molecules.

Food allergy

Plant-derived foods
The main genuine, heat- and pepsin-stable plant-food allergens include: a) Nonspecific Lipid Transfer Proteins (nsLTP). They belong to PR-14, and are typically located near the peel. nsLTP from *Rosaceae* family are highly homologous; b) 2S Albumins (prolamin superfamily) are small storage proteins. They are the major allergen in nuts and seeds. Homology is quite high between cashew and pistachio, sesame and *Brassicaceae*, and reaches 60% between walnut and hazelnut; c) Vicilins (7S globulins) (cupin superfamily) are storage proteins causing allergy to fruits and legumes; d) Legumins (11S globulins) (cupin superfamily) are storage proteins causing allergy to fruits and legumes; e) Gliadins (ω-5 gliadin) (prolamin superfamily) cause wheat allergy.
Molecules involved in cross-reactivity with pollen allergens are heat- and pepsin-sensitive, are in most cases associated with symptoms limited to the oral cavity, and belong to the following families: a) PR-10 (Bet v 1-like) are present in a large number of plant foods. Birch pollen is generally the primary sensitizer; b) Profilin is a plant pan-allergen present in all eukaryotic cells. Primary sensitizers are in general grass or birch pollen.

Animal-derived foods
The main molecular classes involved in food allergy are shown in **table II** (p. 55). All but beta-lactoglobulin and alpha-lactalbumin are heat-resistant.

Main molecules involved in hymenoptera venom allergy

The main molecules involved in hymenoptera allergies are shown in **table III** (p. 56).

Laboratory Methods used for CRD

A technical analysis of the laboratory methods currently available to measure IgE to allergen components goes beyond the scope of this short guide. Essentially, two strategies are employed: a) Singleplex detects IgE specific for single molecular components. A direct knowledge of the patient by the specialist is required. It is a quantitative method; b) Multiplex detects IgE to a large, fixed panel of allergen molecules; the sensitization profile produced may be not necessarily completely associated with clinical symptoms. It is a semi-quantitative method that needs to be correctly interpreted by a specialist.

Recommendations for a correct use of CRD in allergology

The goal is to detect precisely patient's allergy profile in order (a) to prescribe the correct allergen immunotherapy (AIT) in those with respiratory allergy; and (b) to identify the risk of severe allergic reactions in patients with food allergy (9).
A diagnostic algorithm for poly-sensitized patients with respiratory allergy is depicted in **figure 1** (p. 57). The main point is to detect IgE to markers of genuine sensitization to the single allergen sources (pollens, molds, mites, animal allergens, or natural rubber latex) and to cross-reactive molecules. Only genuine reactors will be considered for AIT. In view of the high homology between different cross-reacting allergens (e.g., polcalcins, profilins), detecting IgE to one single representative molecule is considered sufficient to diagnose or rule out sensitization (10,11).
A diagnostic algorithm for food-allergic patients is shown in **figure 2** (p. 57). The clinical risk has to be assessed for every relevant allergen source on the basis of the heat- and pepsin-stability of the sensitizing protein(s).
A diagnostic algorithm for patients showing poly-sensitization to hymenoptera venoms is shown in **figure 3** (p. 57). Again, the goal is to detect the presence of IgE specific for genuine markers of sensitization rather than cross-reactive molecules, in order to prescribe the appropriate venom immunotherapy.

The interpretative comment by the Lab

The experienced pathologist may play a role in the diagnosis of allergic diseases only if he/she receives clinical details along with the request of specific IgE measurement.

Table I *- Main molecules detected in the most important sources of respiratory allergy.*

Source	Major specific allergens	Minor specific allergens	Cross-reactive allergens
grass (*Phleum pratense*)	Phl p1[1] Phl p5[1]	Phl p 2[1] Phl p 4[1] Phl p 6[1] Phl p 11[1]	Phl p7 (polcalcin)[1] Phl p 12 (profilin)[1]
grass (*Cynodon dactylon*)	Cyn d 1[1]		Cyn d 7 (polcalcin) Cyn d 12 (profilin)
birch (*Betula verucosa*)	Bet v 1[1]	Bet v 6[1]	Bet v 2 (profilin)[1] Bet v 4 (polcalcin)[1]
Parietaria judaica	Par j 2 (nsLTP)[1]	Par j 1 (nsLTP)	Par j 3 (profilin) Par j 4 (polcalcin)
olive (*Olea europea*)	Ole e 1[1]	Ole e 7 (nsLTP)[1] Ole e 9 (1-3 beta-glucanase)[1], Ole 5, Ole 6, Ole 10, Ole 11	Ole e 2 (profilin) Ole e 3 (polcalcin) Ole e 8 (polcacin)
cypress (*Cupressus arizonica*)	Cup a 1[1]		
mugwort (*Artemisia vulgaris*)	Art v 1 (defensin-like protein)[1]	Art v 3 (nsLTP)[1] Art v 6	Art v 4 (profilin) Art v 5 (polcalcin)
ragweed (*Ambrosia artemisiifolia*)	Amb a 1 (pectate-lyase)[1]	Amb a 3 Amb a 4 (defensin-like protein) Amb a 6 (nsLTP)	Amb a 8 (profilin) Amb a 9 (polcalcin)
plantain *(Plantago lanceolata)*	Pla l 1[1]		Pla l 2 (profilin)
plane *(Platanus acerifolia)*	Pla a 1[2]	Pla a 2[2] Pla a 3 (nsLTP)[2]	
Dermatophagoides pt.	Der p 1(cistein-proteasi)[1] Der p 2 (NPC2)[1] Der p 23[1]	Der p 3, Der p 4, Der p 5, Der p 6, Der p 7, Der p 8, Der p 9, Der p 11, Der p 14, Der p 15, Der p 18, Der p 21, Der p 24	Der p 10 (tropomyosin)[1] Der p 20 (arginine-kinase)
cat (*Felis domesticus*)	Fel d 1[1] (secretoglobulin)	Fel d 3 (cistatin) Fel d 5 (IgA) Fel d 6 (IgG) Fel d 7 Fel d 8	Fel d 2 (serum albumin)[1] Fel d 4 (lipocalin)[1]
dog *(Canis familiaris)*	Can f 1 (lipocalin)[1]	Can f 5 (kallicrein)[1]	Can f 2 (lipocalin)[1] Can f 6 (lipocalin) Can f 3 (serum albumin)[1]
Alternaria alternata	Alt a 1[1]	Alt a 3, Alt a 4, Alt a 5, Alt a 6[2], Alt a 7, Alt a 8, Alt a 10, Alt a 12, Alt a 13, Alt a 14, Alt a 15	
latex	Hev b1[1], Hev b 3[1] Hev b 5[1], Hev b 6[1]	Hev b 4, Hev b 7, Hev b 9[1], Hev b 11[1], Hev b 12, Hev b 13, Hev b 14, Hev b 15	Hev b 8 (profilin)[1]

[1]Available in singleplex diagnostics. [2]Available only in multiplex diagnostics (ISAC).

Table II *- Main allergens detected in the most important food sources.*

Source	*Major specific allergen*	*Minor specific allergen*	*Cross-reactive allergens*
peach	Pru p 3 (nsLTP)[1]	Pru p 7 (peamaclein)	Pru p 1 (PR-10)[1] Pru p 4 (profilin)[1]
apple	Mal d 3 (nsLTP)[1]		Mal d 1 (PR-10)[1] Mal d 4 (profilin)
hazelnut	Cor a 14 (2S-albumin)[1] Cor a 8 (nsLTP)[1] Cor a 9 (legumin)[1]	Cor a 6, Cor a 10, Cor a 11 (vicilin), Cor a 12 (oleosin), Cor a 13 (oleosin)	Cor a 1 (PR-10)[1] Cor a 2 (profilin)
walnut	Jug r 1 (2S-albumin)[1] Jug r 2 (vicilin)[2] Jug r 3 (nsLTP)[1]	Jug r 4 (legumin)	
brazilian nut	Ber e 1 (2S-albumin)[1]	Ber e 2 (cupin)	
peanut	Ara h 1 (vicilin)[1] Ara h 2 (2S-albumin)[1] Ara h 3 (legumin)[1] Ara h 9 (nsLTP)[1]	Ara h 6 (2S-albumin)[1] Ara h 7 (2S-albumin) Ara h 10 (oleosin) Ara h 11 (olesosin) Ara h 12, Ara h 13, Ara h 14, Ara h 15, Ara h 16, Ara h 17	Ara h 8 (PR-10)[1] Ara h 5 (profilin)
cashew (pistachio)	Ana o 1 (vicili) Ana o 2 (legumin)[2] Ana o 3 (2S-albumin)[1]		
soybean	Gly m 5 (vicilin)[1] Gly m 6 (legumin)[1]	Gly m 7, Gly m 8 (2S-albumin)	Gly m 4 (PR-10)[1] Gy m 3 (profilin)
sesame	Ses i 1 (2S-albumin)[2] Ses i 3 (vicilin) Ses i 4 (oleosin) Ses i 5 (oleosin) Se i 6 (legumin)	Ses i 2 (2S-albumin) Ses i 7 (legumin)	
wheat	Tri a 14 (nsLTP)[1] Tri a 19 (ω-5 gliadin)[1]	Tri a 18 (aglut / isolect) Tri a 20 (γ-gliadin) Tri a 25 (tioredoxin) Tri a 26 e 36 (glutenins) Tri a 37 (α-purotionin) Tri a 30 (α-amilase inhib)[2] Tri a 41, 42, 43, 44, 45	Tri a 12 (profilin)
cow's milk	Bos d 4 (α-lactalbumin)[1] Bos d 5 (β-lactoglobulin)[1] Bos d 8 (casein)[1]	Bos d 2 (lipocalin) Bos d 3 (S100 CBP) Bos d 6 (serum albumin)[1] Bos d 7 (Immunoglobulin) Bos d Lactoferrin[2]	
hen's egg	Gal d 1 (ovomucoid)[1] Gal d 2 (ovoalbumin)[1] Gal d 3 (ovotranferrin)[1]	Gal d 4 (lysozyme)[1] Gal d 5 (livetin)[2] Gal d 6 (YGP42) Gal d 7 (myosin light chain)	
cod fish	Gad c 1 (parvalbumin)[1]	Gad m 2 (enolase) Gad m 4 (aldolase)	
shrimp	Pen i 1 (tropomyosin)[1] Pen m 1 (tropomyosin)[2]	Pen m 2 (arginine-kinase)[2] Pen m 3 (Myosin light chain) Pen m 4 (sarcoplasmic CBP)[2]	

[1]Currently available for singleplex diagnostics. [2]Available only in multiplex (ISAC) diagnostics.

Table III *- main allergenic molecules detected in hymenoptera venoms.*

Source	Allergen	Biochemical name
apis mellifera (honey bee)	Api m 1[1]	phospholipase A_2
	Api m 2[1]	ialuronidase
	Api m 3[1]	acid phosphatase
	Api m 4[2]	mellitin
	Api m 5[1]	dipeptidyl-peptidase IV
	Api m 6	
	Api m 7	cub serin-protease
	Api m 8	carboxilesterase
	Api m 9	serin-carboxipeptidase
	Api m 10[1]	icarapin variant 2
	Api m 11	
	Api m 12	vitellogenin
vespula vulgaris (yellow jacket)	Ves v 1[1]	phospholipase A_1
	Ves v 2	ialuronidase
	Ves v 3	dipeptidyl-peptidase IV
	Ves v 5[1]	antigen 5
	Ves v 6	vitellogenin
polistes dominulus (paper wasp)	Pol d 1	phospholipase A_1
	Pol d 4	serin-protease
	Pol d 5[1]	antigen 5
vespa crabro (hornet)	Vesp c 1	phospholipase A_{1b}
	Vesp c 5	antigen 5

[1]Available for singleplex diagnostics. [2]Available only for multiplex (ISAC) diagnostics.

A series of examples in respiratory or latex allergy are shown in **table IV** (p. 58), while some examples for food allergy are shown in **table V** (p. 59).

In hymenoptera venom allergy, molecular findings should be interpreted in the light of clinical history and of in-vivo and in-vitro results with whole venom extracts. Molecular allergens may confirm sensitization to CCDs. A differential diagnosis between paper wasp and yellow jacket allergy can be afforded if the difference in IgE levels between Ves v 5 e Pol d 5 exceeds 45-50%. Allergen ISAC microarray provides a semi-quantitative measurement of IgE to 112 allergen molecules. It has to be considered a 3rd level analysis, to be used by the specialist to solve doubts and complex cases; the interpretation of the results should be left to the experienced allergologist with special expertise in molecular allergology and is not a duty of the pathologist.

Conflict of interest

The authors declare that they have no conflict of interest.

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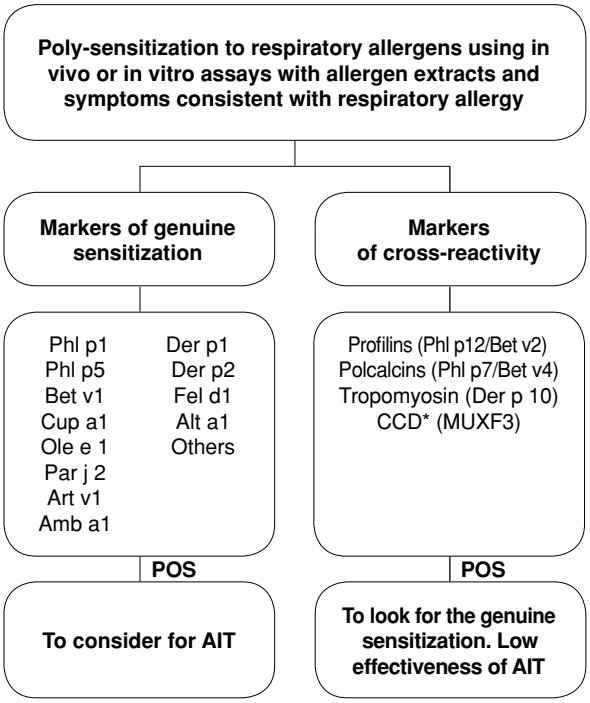
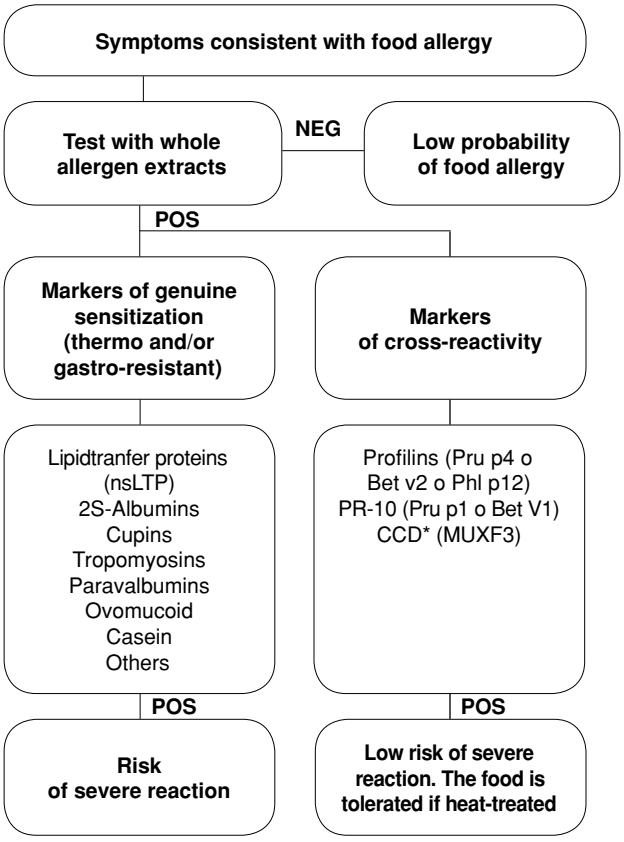
Figure 1 *- General diagnostic algorithm in case of multiple sensitization to respiratory allergens on in-vivo or in-vitro tests with whole allergen extracts. Genuine markers of sensitization and of cross-reactivity have to be chosen on the basis of the positive findings with extracts.*



*The detection of IgE to CCD will be performed only in case of multiple positivity in-vitro.

Figure 2 *- General diagnostic algorithm in case of multiple sensitization to food allergens on in-vivo or in-vitro tests with whole allergen extracts. Genuine markers of sensitization and of cross-reactivity have to be chosen on the basis of the positive findings with extracts.*



*the detection of IgE to CCD will be performed only in case of multiple positivity in-vitro.

Figure 3 *- CRD algorithm in case of positive for both honey bee and vespids using whole allergen extracts.*

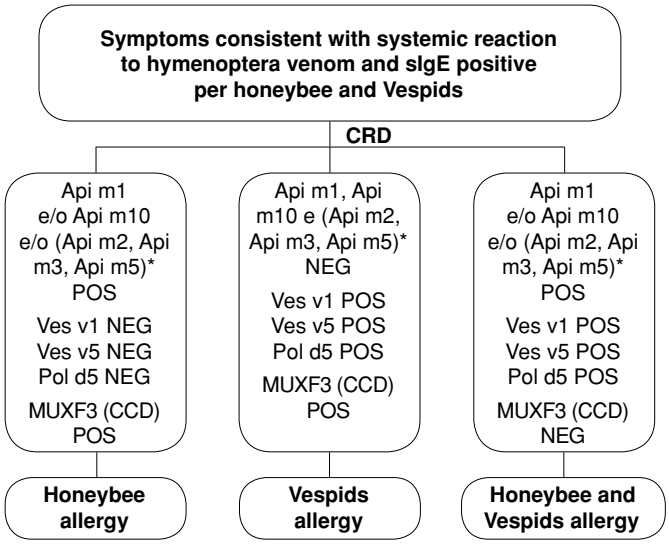


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Table IV - *Some interpretative comments to molecular diagnostics in respiratory and latex allergy.*

Case	**Interpretative comment**
Cross-reacting molecules	
IgE reactivity to PR-10	primary sensitization to birch pollen with cross-reactivity to fruits / vegetable, possibly causing OAS
IgE reactivity to Profilin (Phl p 12/Bet v 2)	profilins are plant panallergens, are frequently cross-reacting and may cause OAS to different plant-derived foods
IgE reactivity to polcalcins (Phl p 7/bet v 4)	polcalcins are pollen panallergens and may be responsible for cross-reactivity between botanically unrelated pollens
CCD	IgE to CCD are cross-reactive and directed against ubiquitous epitopes of plants, invertebrates, latex, and hymenoptera venom. This positivity has no clinical relevance
Seasonal allergens	
single or multiple IgE reactivity to genuine pollen allergens with / without cross-reacting allergens	the patient shows genuine hypersensitivity to the following pollen sources: ... as shown by IgE reactivity to ...
Perennial allergens	
perennial symptoms and positive in vivo / in vitro test with mite extract	Der p 1-2 positive: the test confirms mite allergy; Der p 1-2 negative: mite sensitization not confirmed by the test, but the patient might be sensitized to mite allergens that are currently unavailable for molecular diagnostics
animal allergens	Fel d 1 or Can f 5 positive: primary sensitization to cat / dog; Can f 5 +/Can f 1-: the patient should be able to tolerate contact with female dogs; Can f 1 or other lipocalins positive: in view of possible cross-reactivity, the patient might have symptoms in the presence of different species of animals; Fel d 2-positive: the patient might develop allergic symptoms following the ingestion of pork meat due to cross-reactivity between serum albumins. Serum albumins are partially heat-labile
Aspergillus hypersensitivity	Hypersensitivity to Asp f 1 and/or Asp f 3 is frequent in patients with respiratory symptoms. Hypersensitivity to Asp f 2, Asp f 4, and/or Asp f 6 is more frequent in broncho-pulmonary aspergillosis
latex	Patient monosensitized to Hev b 8 (profilin): this reactivity is clinically irrelevant. No latex-safe procedures needed; Patient sensitized to any other NRL allergen: primary sensitization to natural rubber latex; Sensitization to Hev b 5, Hev b 6 or Hev b 11: possible cross-reactivity to plant foods

Table V *- Some interpretative comments to molecular diagnostics in food allergy.*

Case	Comment
fresh fruits allergy	Sensitization to PR-10 or profilin: allergy caused by pollen / food cross-reactivity, generally associated with local symptoms (OAS). Cooking abolishes allergenicity; Sensitization to nsLTP with history of systemic symptoms: sensitization to heat- and pepsin resistant allergen that may cause severe systemic reactions
nuts and peanut allergy	Hypersensitivity to seed storage proteins or nsLTPs: patient is sensitized to extremely stable allergens that may cause systemic allergic reactions
wheat allergy	Hypersensitivity to Tri a 19 (ω-5 gliadin): patient sensitive to an allergy frequently associated with food-dependent, exercise-induced anaphylaxis
fish allergy	Hypersensitivity to parvalbumin (Gad c 1 or Cyp c 1): in view of the high homology between fish parvalbumins the patient is likely to react to most vertebrate fishes
shrimp / invertebrates allergy	Hypersensitivity to Pen a 1: confirmed allergy to shrimp and other invertebrates; Hypersensitivity to shrimp extract but not to Pen a 1: possible sensitization to shrimp allergens currently not available in the diagnostic kit
cow's milk allergy	Hypersensitivity to Bos d 8: patient sensitized to a heat-stable allergen causing symptoms after boiling; Hypersensitivity to Bos d 4 and/or Bos d 5: patient sensitized to heat-labile allergens; tolerance to cooked food possible
hen's egg allergy	Hypersensitivity to Gal d 1: patient sensitized to a heat- and pepsin- stable allergen. Cooked foods may cause symptoms; Hypersensitivity to gal d 2: patient sensitized to a heat-labile allergen. Tolerance to cooked food possible
allergy to meat	Hypersensitivity to pork meat and to Fel d 2: hypersensitivity to cross-reacting serum albumins (cat-pork syndrome); Alpha-Gal sensitization: this sensitization may cause delayed (4-6 hours) food allergy to red meat; Poultry allergy and Gal d 5 sensitivity (available only on ISAC platform): poultry meat / egg cross reactivity due to sensitization to alpha-livetin

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Immunogenetics of cytokine genes in parthenium dermatitis: a review

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Key words
parthenium dermatitis; delayed type-hypersensitivity; T-lymphocyte; cytokine; immunogenetics; gene polymorphism

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Doi
10.23822/EurAnnACI.1764-1489.40

Summary
Parthenium dermatitis is a chronic immuno-inflammatory, distressing skin disease, and is mediated by activated T-lymphocyte, which is primarily manifested on the exposed sites of the face, neck, hand and flexures. Parthenium hysterophorus is ubiquitous, hence it is difficult to avoid the aero-allergenic antigen parthenin, responsible for the contact dermatitis. The pathogenesis of parthenium dermatitis is characterized by infiltration of T-lymphocytes into challenged skin sites, and the development of a cutaneous inflammation due to altered regulatory network of pro and anti-inflammatory cytokines. Regulation of inflammatory events perpetuated by cytokines continues to complicate efforts to analyze both the function of individual cytokine and the influence of candidate gene polymorphism on expression and disease severity. The genetic polymorphisms in these cytokines are significantly affecting immunological parameters and, subsequently, modulation and polarization of immune responses. This review has focused mainly on understanding of the mechanisms of genetic susceptibility of cytokine genes in this disease and, further, this process is likely to achieve significant advances in the diagnosis and management of parthenium dermatitis.

Introduction

Parthenium dermatitis is a chronic immuno-inflammatory, distressing skin disease, and is caused by activated T-lymphocytes mediated delayed-type hypersensitivity. The alien weed *Parthenium hysterophorus* is ubiquitous, and the commonest cause of plant-induced airborne contact dermatitis (ABCD) in India (1-4). The incidence of parthenium contact dermatitis is increasing considerably year by year, in India and in other parts of the World (5). The parthenium dermatitis reaction is characterized by infiltration of T lymphocytes into challenged skin sites, and the development of a cutaneous inflammation. Subsequently, over its due course, cutaneous infiltration of various type of immune cells leads to an activation of the cellular immune system, with T cells and a diverse range of immune-related cytokines and chemokines implicated in pathogenesis (6,7). This feature continues to complicate efforts to analyze both the function of individual cytokines and the influence of cytokine gene polymorphism on gene expression and dermatitis condition; hence, knowledge of immunogenetics of cytokine genes is of utmost importance in this disease.

Epidemiology

Parthenium dermatitis is an airborne contact dermatitis (ABCD) commonly caused by obnoxius weed *Parthenium hysterophorus*, which is responsible for more than 30% of contact dermatitis in India. This weed achieved a status of global significance as responsible for severe human health issues, such as contact dermatitis and respiratory distress. Apart from this, this invasive

alien weed is considered to be one of the worst plant species for agricultural crops and causes great loss to biodiversity. This aggressive weed at its maturity releases 'parthenin', an airborne antigen to the immediate environment which is a leading cause of airborne contact dermatitis in India (3,8,9). This weed has infested most of rural and waste fields of urban areas, exhibiting the ability to grow prolifically, and in due course invade and adapt to new habitats eventually reducing the number of indigenous plants (8,10). This invasive weed has now become native of more than 20 countries around the World, in five continents (3,9). The pathological condition of parthenium dermatitis, due to various reasons alters the regulatory network of pro and anti-inflammatory cytokines, which leads to cell mediated hypersensitivity (6).

Allergenicity due to *Parthenium hysterophorus*

The aero-allergen responsible for the contact dermatitis is parthenin, which is sesquiterpene lactones in nature, profoundly present in the oleoresin fraction of the leaf, stem, flower and to some extent in pollen of *Parthenium hysterophorus* (**figure 1**). Sesquiterpene lactones are biologically active plant chemicals identified in many plant families in different geographical regions. *P. hysterophorus* contains parthenin, hymenin, coronopilin, hysterophorin and tetraneurin A as major constituents of sesquiterpene lactones (11,12,13). Maishi et al. reported that parthenin, a pungent glycoside, is a major sesquiterpene lactone in *P. hysterophorus* (14). Parthenin is the major constituent of the antigen causing dermatitis in America, Mexico, West Indies and India; it is replaced by hymenin as an antigen in southern Bolivia and central Argentina, and in one population from Texas. Patients with contact dermatitis to *Compositae* plants can also have cross reaction to sesquiterpene lactone; containing other non-*Compositae* plants, however, this cross-reactivity between sesquiterpene lactones does not follow any specific pattern (15,16). Parthenium dermatitis is not reported in some parts of America because of the absence of parthenin in these regions, whereas the dermatitis is severe and causes a major health challenge in Indian subcontinent, where the plant contains large quantity of parthenin antigen (17,18).

Pathogenesis and mechanism of sensitization

The kinetics of delayed type-hypersensitivity response remains biphasic, and manifests as a contact sensitization phase on initial contact with parthenium antigen, and a later elicitation phase. Parthenium dermatitis is an allergen specific-inflammatory disease, in which parthenium antigen, upon contact sensitization to exposed site of skin, leads to a cell-mediated hypersensitivity immune response, that first induces a refractory phase where probably there is no response, and a subsequent induction phase if persistent antigen exposure further continues to sensitize (2,19,20). Indeed, this immune response is mediated by involvement of a series of cellular and molecular mechanisms. Epidermal antigen presenting Langerhans cells, a family of dendritic cells and other cutaneous dendritic cells, transports the allergen from the skin to regional lymph nodes, where it presents the processed antigen to naïve T-lymphocytes (21). During the sensitization phase, contact allergens stimulate epidermal cells to synthesize and release pro-inflammatory cytokines, such as TNF-α and IL-1, which initiates the activation of LC by expression of costimulatory, adhesion molecule and chemokine receptors. Subsequently, they promote LC migration from the site of Ag encounter to the area of T-cell priming in the skin (22). Activation of naïve specific T-cell precursors occurs in the regional draining lymph node, upon presentation of haptenated peptides by cutaneous migrating Langerhans cell. Consequently, T-cell proliferation and differentiation occurs, with production of short-lived effector and long-lived memory T-cells. T-cell activation is a highly regulated process, which requires signals at the site of ongoing allergic contact dermatitis, and regional draining lymph nodes (23-26). Upon contact sensitization with antigens, co-stimulatory MHC molecules, like members of B7 family (CD80 and CD86), CD40 and other molecules, are up-regulated on the surface of cutaneous antigen presenting cells (APCs), and keratinocytes that bind with CD28 receptors on the T-cell surface. Antigen presentation in the absence of costimulatory signals leads to T helper cell clonal anergy, a type of immunologic unresponsiveness characterized by reduced cytokine synthesis, a lack of proliferation, and failure to differentiate into effector cells when reencountered with their cognate antigen (22,27-28). The mechanism of signal molecules gene expression in APC and T-cells is pathologically important in amplifying T lymphocyte-mediated inflammation during contact dermatitis. This alteration of surface molecules on APCs starts incidentally, after contact with antigen and induces T-cell proliferation, cytokine secretion, changes in gene expression, and causes pathogenic skin condition (29,30).

Clinical aspects

Most of the airborne contact dermatitis starts from the eyelids, primarily, affecting the exposed sites of the face, neck and flexures, that are presented with erythema, blistering and intense pruritus, resulting later in skin thickening, hyper pigmentation and development of a leonine facies. Possibly, unexposed sites may also get involved late in the course of the disease, upon antigen sensitization continued specifically. A seasonal variation has an important role, which is observed with flaring and aggravated dermatitis in the summer, corresponding to the growing season of parthenium plants and remission in winter (31-33). Mixed pattern is a combined form of classical and chronic ac-

tinic dermatitis, which manifests as scattered infiltrated scaly papules over exposed site. Classical pattern of dermatitis changes to photodermatitis and photosensitive pruritic lichenified eruption, which presents with discrete, violaceous papules and plaques which develops after many years without seasonal variation (34). Exacerbation of parthenium dermatitis occurs mainly in summer and rainy seasons because of profuse growth of weed parthenium plants, and is prevalent in winter season in southern India during the months of September, October and November, which is maybe due to the increased growth of parthenium following the North-East monsoon showers, as reported by Lakshmi C. (35,36). The skin of the upper eyelids, the retro-auricular and submental areas, which are spared in photodermatitis, are involved in parthenium dermatitis. The dermatitis can become generalized to produce an erythroderma (37). In a recent study, parthenium dermatitis severity scores (PDSS) is found to be a useful tool in determining the severity of the disease, and may be used by clinicians for appropriate scoring of the clinical severity of parthenium dermatitis and monitoring the disease response to therapy. The severity of parthenium-induced dermatitis in sensitized patients depends on the degree of contact hypersensitivity at that time, quantity of antigen, and areas in contact with the patients (38). The effect of seasonal variation also plays an important role on degree of contact hypersensitivity, as the sensitivity to parthenium allergen increases more in summer than winter (39).

Treatment and management

It has been established that corticosteroids are the mainstay of recommended treatment in the parthenium induced contact dermatitis, especially in severe cases. Topical steroids can be used for mild to moderate disease, while severe / extensive dermatitis will require systemic steroids (19,35). Azathioprine acts as an inflammatory cell inhibitor, corticosteroid-sparing agent, has an immunosuppressive and anti-inflammatory effect, and plays an important role in management of chronic parthenium dermatitis, as reported by Verma KK et al. (2006) (40,41).
Azathioprine or other adjuvants like methotrexate or cyclosporine can be used in maintenance doses, and suppress the delayed type hypersensitivity reaction. Along with medication, protection can be given by covering the exposed parts, removal of the patient from the contaminated environment, desensitization methods. Although, complete allergens avoidance from the environment is almost impossible for sensitized patients (42-44). Cyclosporine is also an immunosuppressive with potent anti-inflammatory properties, and has been reported to be effective in the severe condition of parthenium dermatitis. It produces a swift response, and also spares from the side effects of systemic corticosteroid treatment. It suppresses the delayed hypersensitivity reaction, as well as the late phase reaction of type I hypersensitivity (35).

Parthenium hysterophorus is ubiquitous, hence it is difficult to avoid the aero-allergens; the only option is to reduce the quantity of antigen exposure to which patient is already exposed. Exposure to sunlight is also distressing to the parthenium patients, as it exaggerates the disease. Covering of exposed parts of skin and using sunscreen lotions that may serve as barrier creams, can slow down the penetration of the antigens into the sensitized skin (45).

Immune responses in parthenium dermatitis

Parthenium dermatitis is better known as an immuno-modulatory dermatitis, which upon contact sensitization with antigen leads to a cell mediated immune response with the kinetics of sensitization, and an elicitation phase followed by stimulation of naïve T cells. Furthermore, during the sensitization phase, contact allergens stimulate epidermal cells to synthesize and release pro-inflammatory cytokines such as TNF- α and IL-1, which in turn promote Langerhans cells migration from the skin. The challenged skin sites reaction serves as a repository for proliferation of activated T-lymphocytes, that eventually produce effectors and memory cells which lead to development of cutaneous inflammation, and endows with rapid and specific responses upon re-exposure of sensitized antigens (20,46,47). The mechanism of pathogenesis involves a complex, intricate regulatory network of inflammation mediators, T regulatory and pro and anti-inflammatory cytokines released by various immune modulator cells (48). In normal condition, these cytokines cross regulate each other, and a balance exists between these modulatory molecules (49). Depending upon the kind of allergen encounter and cytokine milieu, polarization of naïve T-helper cells happens in direction of TH1, TH17 or TH2 cells type immune responses (50,51,30). During the entire inflammatory reaction, cytokines released by various immune cells function as communicators between different cells. Altogether, the type of immune response is determined and directed by all the existing factors after antigen sensitization (52,53).
T reg cells contribute to the control of allergen-specific immune responses through multiple mechanisms: suppression of antigen-presenting cells that support the generation of TH1 and TH2 effector cells, and remodeling of resident tissue cells. Immune system dysregulation and T helper cells play a key role in eliciting and maintaining inflammation in the skin during contact sensitization with antigen. Mechanism of enhancing skin inflammation by aero-antigen includes expansion and migration of skin-homing T cells, and inhibition of T reg cell immunosuppressive function (54).

Cytokine profiling in parthenium dermatitis

The immune response is regulated by a highly complex and intricate network of control elements; a dynamic balance ex-

ists between proinflammatory cytokines and anti-inflammatory components (48,55). Polarization of T-helper (TH) lymphocytes into functional TH1 and TH2 subsets is one of the main factors that determine the direction of the balance between pro- and anti-inflammatory cytokines (50,56). A number of cytokines, known collectively as pro-inflammatory cytokines because they accelerate inflammation, also regulate inflammatory reactions, either directly or by their ability to induce the synthesis of cellular adhesion molecules or other cytokines in certain cell types. Regulation of T-cell activation by the anti-inflammatory cytokines is a crucial early control element in the process of inflammation of skin (51,57). Our previous studies on parthenium dermatitis showed elevated circulating levels of proinflammatory cytokines such as IFN-γ, IL-2, TNF-α, IL-6, IL-8, IL-17, and lower levels of anti-inflammatory cytokines such as IL-10 and TGF-β in patients compared with controls, Akhtar et al. (2010). This suggests that the lower circulating level of IL-10 and TGF-β (an anti-inflammatory and immunosuppressive cytokine) might be insufficient to counter- regulate the proinflammatory signals which lead to a hypersensitivity immune response (2,4).

Immuno-genetics of cytokine genes polymorphisms

There are a number of factors which affect the levels of cytokine production among different individuals. These include gene transcription stability, post translation modification, protein intracellular stability, and the export of cytokine to the extracellular environment. Besides this, the genetic polymorphism of these cytokine genes in gene regulatory and protein coding regions majorly affects cytokine production in different individuals (7,58). The pathogenesis of parthenium dermatitis is considered to be an immunologically mediated process. Due to various reasons, altered regulatory network of pro and anti-inflammatory cytokines leads to cell mediated hypersensitivity. Regulation of inflammation events perpetuated by cytokines acts in a highly complex coordinated network, in which they induce or repress their own synthesis as well as that of other cytokines and cytokine receptors. This feature continues to complicate efforts to analyze both the function of individual cytokines and the influence of cytokine gene polymorphism on gene expression and disease (59,60).

Based on various parameters, previous reports suggested that parthenium dermatitis pathogenesis involves cell-mediated hypersensitivity immune response, and happens due to imbalance in various pro and anti- inflammatory and T reg cell cytokines. Among the number of risk factors leading to allergy, the interaction of genetics of an individual with environment is one of the affecting significant parameters. The functional genetic alterations in structure of these cytokine genes are among a number of factors which influence the variation in systemic cytokine levels. The genetic polymorphisms in these cytokine genes are significantly affecting parameters, which influence the inter-individual differences in cytokine levels and determine the balance between these, and direct the kind of human immune response. In many diseases, these polymorphic cytokine gene regions have been found as susceptibility factors (61,62).

In a recently published study, we have analyzed IFN-γ (+) 874 A > T and IL-10 (-) 1082 G > A and (-) 819 C > T single nucleotide polymorphisms in parthenium dermatitis cases vs. control subjects. The study showed that the IFN-γ (+) 874 A > T SNP genetically does not justify the high serum levels of IFN-γ in parthenium dermatitis patients in comparison to healthy controls, whereas the lower producing genotypes due to IL-10 (-) 1082 G > A and (-) 819 C > T SNPs remain in step with the prevalence of low serum IL-10 in parthenium patients, and these genotypes genetically predispose to this disease. The intermediate IL-10 producing genotypes due to these SNPs in IL-10 gene, provide resistance to the carriers of these genotypes for not developing the disease (7,63) (**table I**).

In earlier reports, altered levels of TNF- α have been found involved in pathogenesis of psoriasis. There are numerous polymorphisms found in TNF-α promoter region, which might modulate TNF-α expression. The single nucleotide polymorphism at position (-) 308 G/A has been found to be a functional and genetic predisposition to various diseases, like liver cirrhosis, rheumatoid arthritis, inflammatory bowel disease, etc. This polymorphism has also been reported in asthma and other allergic diseases like occupational chronic irritant contact dermatitis (CICD) and psoriasis (64-66).

The prevalence of high TH1 cytokines (IFN-γ, IL-2, pro- inflammatory IL-6, IL-8, IL-17, TNF-α) and declined levels of TH2 cytokines (IL-10) and T reg cytokine (TGF-β1) reported by Akhtar et al., suggests that an insufficient amount of anti-inflammatory and immunosuppressive cytokines might unable to downregulate the TH1 cytokines. Thereby, their levels might remain high in patients (2). Khatri et al. (2013) found that the SNPs IFN-γ (+) 874 A > T and TNF-α (-) 308 G > A are not associated with the disease. Therefore, high serum levels of IFN-γ and TNF-α in patients are not genetically in step with the studied SNPs in these genes (67). The alleles at both the loci did not lead to any association with the disease, but the low IL-10 producing genotypes such as AA and TT, created a 2.01 and 3.45 times higher risk respectively for patients for developing the parthenium dermatitis, whereas the intermediate IL-10 producing GA and CT genotypes at these loci provided 54% and 67% resistance respectively to the individuals for not developing the disease. The low TGF-β1 producing genotypes CC at this locus were prevalent in parthenium patients compared to controls, but upon statistical comparison the p-value remained little far from value of significance. In conclusion, concordance between

Table I *- Cytokine and their genetic polymorphism studies in parthenium dermatitis.*

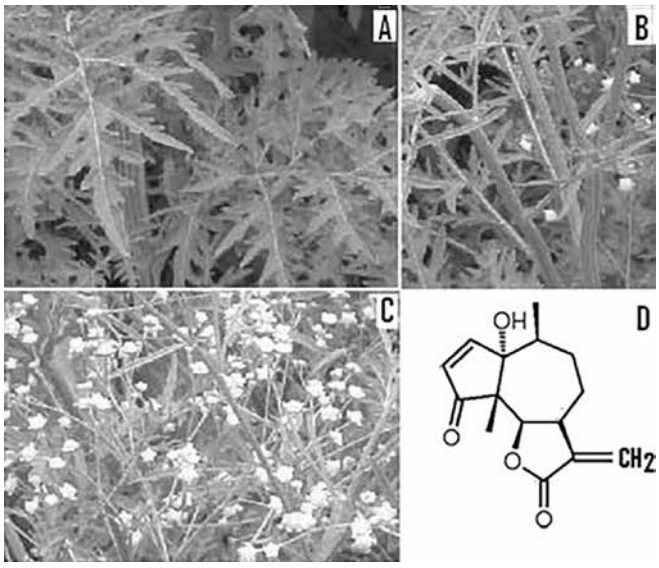
Source and related parameter	***Significance patients vs. control***	***References***
TH1 type cytokine IL-2, IFN-γ	significantly increased compared to control	Akhtar N et al. (2010) Contact Dermatitis
TH17 type cytokine IL-17	significantly increased compared to control	
TH2 type cytokine IL-4, IL-10	only IL-10 significantly decreased	Akhtar N et al. (2010) Clin Chem Acta
T reg type cytokine TGF-β	significantly decreased	
IL-6, IL-8, TNF-α	significantly increased compared to control	Akhtar N et al. (2010) Contact Dermatitis
Genetic polymorphism	Risk assessment	
IFN-γ (+) 874 A > T	no association	Khatri R et al. (2011) BJD
IL-10 (-) 1082 G > A and (-) 819 C > T	2.01 and 3.45 times more risk	
TNF-α (-) 308 G > A	no association	Khatri R et al. (2014) IJD
TGF-β1 (-) 509 C > T	associated	unpublished
IL-4 (-) 590 C > T	no association	unpublished

low TGF-β1 producing CC genotypes along with low serum levels of TGF-β1, suggested the association of this locus with the disease, which needs further validation in larger sample size.

Conclusions

Several studies to date have been reported to analyze the influence of gene polymorphisms on various cytokine gene expression and disease conditions. Cytokines are communicating molecules between cells of immune system, and express a critical role in polarization, amplification and modulation of immune responses, and determine which effector mechanisms are to be employed in response to immune challenge. Our studies on parthenium dermatitis suggest the prevalence of high serum IFN-γ in patients, but the IFN-γ (+) 874 A > T transition genetically does not contribute to the parthenium dermatitis. The small sample size may be one of the limitations, and a larger cohort study may provide conclusive association of genetic predisposition of IFN gene. In contrast, low IL-10-producing genotypes in patients is associated with a high prevalence due to IL-10 (-) 1082 G > A, and (-) 819 C > T polymorphisms. The concerned IL-10 genotypes at these loci show a strong genetic predisposition to parthenium dermatitis in Indian cohort, and function as risk factors. Mostly, healthy individuals possessed intermediate IL-10 producing GA and CT genotypes with a statistically significant difference from dermatitis patients. In our recent study, no genetic endowment

Figure 1 *- Parthenium hysterophorus. A, leaves; B, mature plant; C, flowers; and D, chemical structure of parthenin antigen. Figure from our published paper.*



of TNF-α (-) 308 G > A polymorphism was found in pathogenesis / susceptibility associated to parthenium dermatitis, even with high level of TNF-α. A thorough prospective analysis in a higher number of subjects is required to understand the role of

TNF- α genetic variation in pathogenesis of parthenium disease. The reports in this review suggest that even though IFN-γ and TNF-α gene polymorphisms are not associated with parthenium dermatitis, genotyping marker in disease condition is considered to be potentially important. Thus, this review enlightens the important role of immunogenetics in parthenium dermatitis. Further continued advances in molecular genetics and in high-throughput of genotyping of cytokine genes are likely to achieve significant advances in the diagnosis and management of parthenium dermatitis.

Conflict of interest

The authors declare that they have no conflict of interest.

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Local anesthetics allergy: who should be tested?

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Key words
allergy; asthma; drug hypersensitivity reaction; local anesthetics, skin test

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Doi
10.23822/EurAnnACI.1764-1489.38

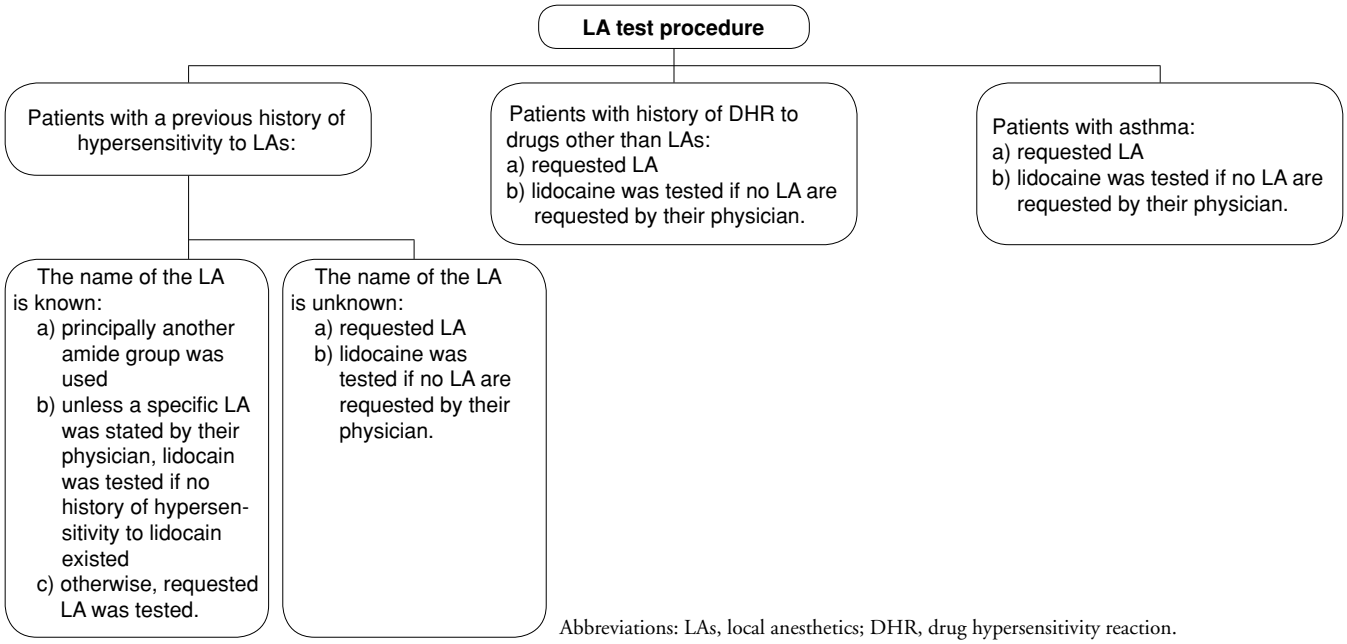
Summary
Objective. *To document the test results of patients referred to our clinic for testing with local anesthetics (LAs) in real life conditions and provide data related to the necessity of these tests.* ***Methods.*** *All consecutive subjects who were referred to be evaluated for LA allergy during a two-year follow up were included in the analysis. All subjects underwent skin prick / intradermal tests followed by a subcutaneous provocation test with the LAs tested.* ***Results.*** *A total of 228 subjects were included. The main referral reason was the presence of a history of drug hypersensitivity reaction (DHR) to drugs other than LAs (n = 128, 56%), whereas a history of LA allergy constituted the second most common referral reason (n = 64, 28.1%). In the majority of cases (n = 39, 60.9%), the culprit LA was not known by the patients. Asthma was the third most common referral reason, presented in 49 cases (21.5%). Ten cases had positivity to the tested LA in skin testing / challenges. Nine out of 10 patients had a history of DHR to drugs other than LA, whereas 5 of them had also a history of DHR to LA. Six of the 10 patients had a history of multiple DHR. None of the asthma patients without any DHR history were positive in the LA tests. Eight out of 10 cases who underwent skin testing / challenge with an alternative LA, tolerated the alternative LA.* **Conclusion.** *The most common referral reason for testing with LA was a history of DHR to drugs other than LAs, whereas asthma was the third most common referral reason. Patients with a history of multiple DHR may be considered for testing with LAs. Asthmatics and those with other allergic diseases without a history of drug / LA allergy do not need to be tested with LA.*

Introduction

Following their first application in the late 19th century, local anesthetics (LAs) have been widely used in the areas of dentistry, ophthalmology, obstetrics and gynecology, and for other minor surgical procedures (1). Since then, several cases with undesired side effects have been reported (2-8). However, the problem with the "undesired effects" of LAs is the miscalling of some symptoms as "allergy" because of the indistinct nature of the symptoms. The incidence of true allergic reactions to LAs is lower than 1% (2-5). It's shown that pharmacological, toxic, pseudo-allergic or autonomic reactions are the main reasons of non-allergic reactions (2-8).

Guidelines recommend that LA testings should be performed in patients with a history of LA hypersensitivity (9-11). However, in our clinical experience, we noticed that not only patients with a history of LA allergy, but also those with asthma without a history of any drug allergies, and patients with a history of drug hypersensitivity reactions (DHR) to drugs other than LAs, were referred to be evaluated for a possible allergy to LAs before a minor surgical procedure. In our daily routine, these cases have to undergo skin testing with the requested LAs, in order to relieve the anxiety of both the patients and the referral physicians, despite the fact that no real indication exists. However, we importantly found that the majority of

Figure 1 *- Test and evaluation protocol for LAs.*



Abbreviations: LAs, local anesthetics; DHR, drug hypersensitivity reaction.

these cases had negative skin tests and challenges, which makes performing these tests unnecessary for the evaluation of these groups. Therefore, we designed an observational real life study, aiming to define the groups of patients who referred to our allergy clinic for testing with LAs and the results of these tests, in order to define the characteristics of the patients who should actually be referred for tests with LAs. We hypothesized that it is not necessary to perform many of the LA tests.

Materials and methods

This observational study was designed prospectively, and all consecutive patients who were tested with LAs for any reason were enrolled to the study for a two-year period. The study was approved by Ankara University Ethics Committee. Patients who were unwilling to participate in the study were not included in the analysis. The demographics of the patients were recorded from case files. The following information was also recorded for each case: referral reason, presence of any allergic disorders and other diseases, any history of LA allergy and/or DHR to drugs other than LAs. In the presence of a history of allergy to LAs, the name of the responsible drug(s) and type of reaction(s) were also recorded. Thus, the patients who were tested for LA allergy were categorized under 3 groups:

1. patients with a history of hypersensitivity reactions to LAs
2. patients with a history of DHR to drugs other than LAs without allergy to LAs
3. patients with asthma.

Drug hypersensitivity related reactions were immediate reactions (urticaria / angioedema, bronchospasm, laryngeal edema, rhinitis, and systemic anaphylactoid reactions involving hypotension, laryngeal edema, bronchospasm and/or shock) and non-immediate reactions (maculopapular eruption, fixed drug eruption, photosensitivity, contact dermatitis, and other reactions) to a prescribed drug. Patients with a history of immediate or nonimmediate type reaction to any kind of drug and those tested for DHR, were enrolled in the study.

Allergologic workup

An algorithm recommended by the ENDA/EAACI Drug Allergy Interest Group was applied in our study (11). Antihistamines as well antidepressive therapy were stopped at least one week prior to skin tests with LAs. LAs without vasoconstrictors were used for all tests in order to avoid false negativity. The following protocol was applied in the selection of LAs to be tested:
All patients underwent skin testing including a skin prick test (SPT) and intradermal test (IDT), followed by a subcutaneous provocation test (SCT) with tested LAs. The IDT was performed when SPTs were negative. Positive (histamine chloride 1 mg/mL) and negative (0.9% sodium chloride) controls were applied on the anterior side of the patients' forearms. Tests were firstly performed by undiluted skin prick tests; if negative, they were followed by intradermal tests using 1/100 and 1/10 dilutions. The positivity of the skin test was established when the

mean wheal diameter was at least 3 mm greater than the negative control for SPT, and at least 5 mm greater for IDT. In patients with negative skin testing, drug provocation tests with increasing subcutaneous doses (0.1 ml and 1 ml) at the lateral surface of the patients' arms, were conducted. Local findings around the injection site, general symptoms, and vital signs were observed for up to 30 minutes (11-13).

Statistics

The statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Numeric values with normal dispersion were expressed as means ± SD, whereas abnormal dispersed variables were given as median values (min-max). Categorical variables' values were given as n (%). When comparing dichotomous independent variables, chi-square of Fisher's exact test was used. The p value of < 0.05 was considered to be significant.

Results

The patients

The study included a total of 228 patients. The mean age was 42.2 ± 1.3 years, range: 16-74 years. Most of the patients were female (77.6%) (**table I**). The most common referral reason for testing with LA was a history of DHR to drugs other than LAs (n = 128, 56.1%), whereas a history of LA allergy constituted the second most common (n = 64, 28.1%), and asthma was the third most common referral reason (n = 49, 21.5%).
Considering the cases with a history of DHR to LAs, the most common clinical symptoms were urticaria (n = 21, 32.8%) and dyspnea (n = 13, 20.3%) (**table II**). In the majority of cases (n =39, 60.9%), the responsible LA was not known by the patients. Among the identified LAs, articaine was the most commonly (n = 11, 17.2%) reported one.

Test results and characteristics of positive response

Prilocaine was the most commonly (n = 125, 46.8%) tested LA based on referral physicians' requests, which was followed by mepivacaine in 83 cases (31.1%) (**table III**). Ten out of 228 cases (4.3%) had positivity to LA in skin testing / challenges. Considering the subgroups, 9 (90%) out of 10 patients had a history of DHR to drugs other than LAs, whereas 4 of these 9 patients also had a history of DHR to LA. Of the 218 patients without test positivity, 170 (78%) had drug allergy history. Six (60%) of the 10 patients who had positive test results, had a history of multiple drug hypersensitivity. Of the 218 patients without test positivity, 71 (32%) had multiple DHR history.

Table I *- Demographics and disease features of the study population.*

Variable	
study population, n	228
female / male, n (%)	187/41 (82%)
age (years ± SEM) (min-max)	44.22 ± 1.28 (16 - 74)
atopy, n (%)	22/91 (24.1%)
Referral reason n (%)	
asthma	49 (21.5%)
urticaria	20 (8.8%)
history of other allergic diseases	22 (9.6%)
history of any drug allergy, n (%) history of multiple drug allergy, n (%)	179 (78.5%) 77 (35.3%)
history of drug reaction with LA, n (%)	64 (28.1%)
history of drug allergy other than LA, n (%) antibiotics NSAIDs radiocontrast agent general anesthetics other	128 (56.1%) 66 (28.9%) 57 (25%) 4 (1.7%) 7 (3.1%) 16 (7%)

Table II *- Documentation of LA hypersensitivity in 64 cases.*

Variable	**n (%)**
Suspected LA	
articaine (Ultracaine 2%®) lidocaine (Jetocaine simplex®) prilocaine (Citanest 2%®) mepivacaine (Isocaine 3%®, Safecaine 3%®) unknown	11 (17.2%) 6 (9.4%) 3 (4.7%) 1 (1.6%) 39 (60.9%)
Manifestation of drug reactions	
urticaria / angioedema, n (%)	21 (32.8%)
lower airway, n (%)	13 (20.3%)
rhinitis, n (%)	8 (12.5%)
CVC, n (%)	7 (10.9%)
anaphylaxis, n (%)	7 (10.9%)
other, n (%)	7 (10.9%)

Comparison of these groups according to their DHR history and test results showed no statistically significant difference. None of the asthmatic patients without any DHR history had

Table III *- LAs used in the drug tests in the study population.*

		Adjuvants			
Local anesthetics	**Commercial products**	**adrenaline**	**sodium metabisulfite**	**methyl- Paraben**	**Number of the patients tested**
mepivacaine	Isocaine 3%® Safecaine 3%®	-	-	-	83 (31.1%)
prilokaine	Citanest 2%®	-	-	+	125 (46.8%)
lidocaine	Jetocaine simplex®	-	-	-	45 (16.8%)
articaine	Ultracaine 2%®	-	-	+	8 (3%)
bupivacaine	Marcaine 0.5%®	-	-	-	6 (2.2%)

positive LA test result. Apart from other drug allergies, associated allergic conditions such as asthma, chronic urticaria, food allergy, bee venom allergy and other allergic diseases were not found to be common in patients who had positive SPT and/or SC provocation tests results.

Lidocaine (n = 4) was the most common LA exhibiting positivity among 10 patients, which was followed by prilocaine in 3 and mepivacaine in 3 patients (**table IV**). All drug reactions manifested within the first hour following LA application. Only 3 cases showed positivity in skin tests, whereas

Table IV *- Characteristics of patients with positive tests to LA.*

Age and gender	**Atopy**	**Presence of allergic diseases**	**History of drug hypersensitivity other than to LA**	**History of hypersensitivity to LA**	**Tested drug**	**Characteristics of positive test**	**Result of testing to find alternative LA**
36, f	negative	asthma	yes (analgesics)	yes (articaine)	lidocaine	hypotension 1 cc SC challenge	mepivacaine: negative
54, f	negative	chronic urticaria	yes (not known)[1]	yes (not known)	lidocaine	dyspnea 0.1 cc SC challenge	mepivacaine: negative
46, f	nd[2]	no	yes (not known)	yes (not known)	prilocaine	rhinitis and pruritus 1 cc SC challenge	nd
44, m	yes	venom allergy	no	yes (not known)	mepivacaine	skin test positive 1/100 ID	prilocaine: negative
36, f	negative	no	yes (not known)	yes (prilocaine)	mepivacaine	facial flushing and pruritus 0.1 cc SC challenge	lidocaine: negative
52, f	nd	no	yes (not known)	no	prilocaine	skin test positive 1/100, ID	mepivacaine: negative
51, m	positive	asthma	yes (antibiotic and analgesic)	no	prilocaine	skin test positive (1/1 prick)	mepivacaine: negative
55, f	nd	asthma	yes (antibiotic)	no	mepivacaine	laryngeal edema pruritus 1 cc sc challenge	nd
58, f	positive	food allergy	yes (antibiotic)	no	lidocaine	dyspnea 0.1 cc sc challenge	mepivacaine: negative
44, f	nd	no	yes (analgesics)	no	lidocaine	laryngeal edema 0.1 cc sc challenge	marcaine: negative

[1]No specific drug name is available. [2]Not done.
Abbreviations: f, female; m, male.

the remaining cases were positive on subcutaneous challenges (**table IV**).
These positive cases were tested with another amide group LA in order to find a safe alternative and all of these tests were found negative (**table IV**).

Discussion

In this study, we tested all cases that were referred to an allergy clinic for LA testing. The most common referral reason was a history of DHR to drugs other than LAs. Our results showed that unless they had a positive history of allergy to a certain LA, patients with asthma were not suitable candidates for drug tests with LA. We also showed that there was a potential to have positive LA tests in patients with multiple DHR.
We recorded all cases who underwent drug tests (skin tests / SC challenges) with LAs for two years. The patients were mostly referred by their physicians because of DHR to drugs other than LAs. Multi-drug allergy is regarded as a risk factor for LA allergy. Patients with allergy to other drugs, and those who had reactions to general anesthetics, are regarded to be at a high risk for LA allergy (14,15). However, several studies suggest that only the previous appearance of unexpected adverse reactions following the administration of LAs is regarded to be a risk factor of a similar or even more severe reaction after further exposure to the same agent (6,9,16). Among the patients who had positive test results in our study, 60% had multi-drug allergy. Statistical significance was not reached, although the history of multiple drug allergies was more frequent in the patients who had test positivity. Though speculative, a multiple DHR history may be a possible risk factor for LA allergy. A larger cohort might be needed to determine this potential risk.
It is reasonable that patients with a history of any drug allergy are referred for testing with LA before a local procedure is performed, in order to be sure about the safe use of LA. However, in asthma patients with no history of DHR, there is no clear association between the use of LAs and the development of hypersensitivity reactions. Only a few case reports indicated that local anesthetics, particularly in nebulized forms, may induce bronchospasm (17,18). In contrast to the bronchospasm effect of LAs, nebulized lidocaine has also been successfully used in the treatment of persistent cough (19). In our study, asthma patients with no history of DHR did not have any positivity in skin tests and challenges. Three of the patients with positive tests with LA had an asthma history. However, the main characteristic of a positive test in these patients was not pulmonary symptoms. The patients also had a DHR history. Therefore, our results suggest that performing skin tests with LAs as a prescreening tool before minor surgery provides no benefit for the patients. Moreover, these tests are time consuming and costly. Supporting our results, in the study of Berkun 236 patients with histories of either adverse reaction to drugs, anaphylactic reactions, food allergy or atopy, but no history of reactions to local anesthetics, skin prick testing and provocative dose challenges, showed no positivity (6).
A recent meta-analysis confirmed that real allergies to LAs are exceptionally rare, accounting for less than 1% of patients with a history of symptoms which resemble allergy (20). Concomitantly administered agents (preservatives, antiseptic preparations, non-steroidal anti-inflammatory drugs, antibiotics), as well as the materials and equipment with which patients may have been in contact during procedures, should be considered as a possible cause of immediate type reactions (21-23). We lack evaluation and testing of other agents like metabisulfides, parabens and latex, which are commonly used during these minor surgical procedures. Also, epidemiologic studies evaluating the frequency of LA allergic reactions and their causes have been difficult to conduct owing to the fact that the main agent responsible is usually not known in most cases, which was also true in our cases. Therefore, in our study diagnostic workup for LA allergy was not performed in patients with a history of hypersensitivity reactions caused by LAs. However, despite this limitation, the important outcome at the end of the test procedure is that almost all patients found an LA that they can use safely.
Positive cases were challenged with other amide containing LAs, in order to find a safe alternative (**table IV**). The cross-reactivity patterns of LAs are not well reported. The pattern between ester-type LAs is generally well established, whereas that between amide anesthetics is not well known. Cross-reactivity between lidocaine and mepivacaine has been reported (25,26). In our series, patients with positive tests with lidocaine showed a negative response to mepivacaine. In addition, a patient who presented with an allergic reaction to articaine, tested positive on SC provocation test to lidocaine and tolerated mepivacaine favorably (**table IV**). Another case of hypersensitivity to prilocaine with positive tests to mepivacaine on SC provocation tests, showed favorable tolerance to lidocaine (**table IV**). Finally, we were able to find safe alternatives for all cases with a positive test to LA. These findings suggest that cross-reactions may not always occur among amide group LAs, and that allergic reactions may be associated with different antigenic epitopes. This situation shows that another amide chain containing drug can be used as an alternative for LA testing. According to these findings, mepivacaine may be a viable alternative that gives reliable results and could be used for testing in patients with a history of DHR.
In our series, only 3 cases had skin test positivity. Importantly, one previous report yielded a 97% negative predictive value for SPTs and intradermal tests (24). However, in our series, 7 cases who had negative results in skin tests had positivity in the SC challenges. The reactions observed in the SC challenges of our series were more subjective symptoms such as dyspnea and la-

ryngeal edema, which were not confirmed by a spirometry but reported to be positive by the physician who commented on the test. Epinephrine content can not explain these symptoms either, as we avoided its use. Importantly, skin prick test positivity shows an underlying IgE mediated pathophysiology; however, SC challenges do not. Therefore, we cannot say that these reactions are IgE mediated, and there is a possibility that these cases were not really allergic.

In conclusion, high numbers of patients that were suspected to have LA allergy were referred for evaluation to find a safe LA that can be used. However, most of these tests were found to be unnecessary, and to be time and money consuming. Patients with controlled asthma do not need to be tested routinely with a LA. Patients with a history of multiple DHR seem to be suitable candidates for testing with LAs. However, patients with a prior history of LA hypersensitivity should definitely undergo LA testing. Attention should be paid to collaboration between allergologists and the physicians administering local anesthesia. Prior records related to the undesired effects of LAs should also be kept and given to the patients.

Conflict of interest

The authors declare that they have no conflict of interest.

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Systematic investigation for underlying causes of recurrent infections in children: surveillance of primary immunodeficiency

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Key words

recurrent infection; primary immunodeficiency; children; confirmatory diagnostic studies; clinical patterns, management

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Doi
10.23822/EurAnnACI.1764-1489.39

Summary

Recurrent infections seem to be a common complaint in children who are referred to general practitioners' and pediatricians' offices. Detection of primary immunodeficiencies (PID) etiology is very important for achieving appropriate diagnosis and treatment of these patients. The absence of appropriate treatment could lead to subsequent complications, in a hospital inpatient and/or outpatient settings. This study was performed in a group of children with recurrent infections to identify patients with underlying PID. A cross-sectional study was designed to evaluate the final clinical diagnosis obtained in 100 pediatric patients with a history of recurrent infections referred to Children's Medical Center, Tehran, Iran, during one year (2011-2012). History taking and physical examination, complementary laboratory tests including immunological investigations were done to confirm the main causes of disease according to our previously published stepwise approach to recurrent infections. Among all studied patients, 21% (11 males and 10 females) were diagnosed to have PID. Parental consanguinity ($p = 0.001$) and soft tissue infections ($p = 0.004$) were significantly higher in PID group, comparing to other causes of recurrent infections. Gender and location of infections were also linked to the type of PID including antibody deficiency, combined immunodeficiency and phagocytosis disorders. The real rate of PID as a cause of recurrent infection appears to be much higher than what is generally considered in a selected group of pediatric patients; so, following the suggested stepwise guideline can improve timely diagnosis and appropriate treatment of these patients.

Introduction

Primary immunodeficiency diseases (PIDs) are a heterogeneous group of inherited disorders associated with infectious and non-infectious complications (1). Infectious diseases occur in children more frequently than adults (2-5). Normally, a child might suffer from infectious episodes between 6-15 times per year (6,7). This range of infectious events depends on many predisposing factors (2,8). The most important promoting elements are age, exposure to other children in school or daycare units (9,10), passive smoking, inadequate nutrition, living in an air-polluted area, atopy, anatomical defects and other chronic underlying disorders (11).

Therefore, a challenge exists for practitioners to verify the abnormal pattern and unusual type of the infection (12), and to make a decision about the necessity of further evaluation for finding more serious underlying conditions such as immunodeficiency (13-15).

To achieve this goal, starting from 20 years ago, diagnostic aids such as "10 warning signs of PID" have been designed (16,17), that were based on patients' medical history and physical examination (7,8). Although these guidelines are so applicable and useful in the diagnosis of PID in both children and adults, further development and refinement of the suggested warning signs show that reliance on these signs cannot identify all patients with PID (3,15,16).

Since early diagnosis and treatment of PID are very important (18,19), it is necessary to know the proportional rate of PID among the children suffering from recurrent infections (20). However, this rate varies among different settings and different ethnicities. Early diagnosis can improve long-term quality of life and prevent secondary complications of PID (9,12). Improving the awareness of pediatricians about these facts could be helpful in better evaluation and management of the condition (21,22). Although there are some important reports on registries of PID patients in Iran and other countries, there is no clinical study on children with recurrent infectious diseases, to classify them into diagnostic subgroups and show the applicability of a stepwise approach to the recurrent infection. Furthermore, there are only a few similar articles in this field all over the world (16,20,23). This survey was designed to identify the frequency of underlying primary immunodeficiency among pediatric patients with recurrent infection.

Materials and methods

Patients

A cross-sectional prospective study was designed. Patients were selected at the main pediatric tertiary hospital, "Children's Medical Center affiliated to Tehran University of Medical Sciences, Tehran, Iran" from November 2011 to November 2012. Patients younger than 14 years old that were referred to the emergency unit, the general pediatric clinic, the immunology, allergy and infectious diseases outpatient clinics., along with the patients admitted to the infectious, gastroenterology, intensive care unit (ICU) and immunology wards, were enrolled. Patients with a history of recurrent or chronic infection and also cases with a serious infectious complication were included to the next step. There is a family medicine for each patient in Iran, and each episode of the medical event should be documented by corresponding family physicians before referring to the third level hospital. Thus, we obtained data of all episodes of infections in the studied patients by reviewing these documents. The Ethics Committee of the Tehran University of Medical Sciences approved the project. Written informed consent was obtained from all the cases and/or their parent(s).

Clinical evaluation

Recurrent infection was defined as a history of at least two severe infections in a year, three or more bacterial respiratory infections (e.g., sinusitis, otitis media, and bronchitis) in one year, or the requirement of antibiotics for two months / year. Severe / serious infections were also considered as grave infections, including those with persistent fever or confinement to bed for a week or more, failure to respond to oral antibiotics and/or the need for intravenous antibiotics or hospitalization, infections with an unusual pathogen, unusual complications (e.g., mastoiditis, pleural effusion, abscesses), or persistent laboratory abnormalities (e.g., prolonged leukocytosis, elevated erythrocyte sedimentation rate [ESR] or C-reactive protein [CRP], persistent imaging abnormalities) (24).

A detailed itemized questionnaire, including whether or not there was a family history of recurrent infection or unexpected death, consanguinity of parents, age at the time of first infection and current age, physical examination details, the types and severity of infections, and reports of para-clinical evaluations (e.g. laboratory tests and imaging and pathological reports of patients), was filled for all enrolled patients.

Furthermore, based on the predominant presentation and infectious location of each patient, we grouped the infections into 6 main categories including upper respiratory infections (25), lower respiratory infections (26-28), gastrointestinal infections, skin infections, soft tissue infections, and severe or life-threatening infections (meningitis, sepsis, osteomyelitis) (23,29,30).

Laboratory tests

From all individuals, a 5 milliliter blood sample was taken for measuring complete blood count (CBC), ESR, CRP; then, according to each medical history and a previously published stepwise flowchart of approach to recurrent infections, complementary diagnostic laboratory tests were done (6), such as serum immunoglobulin levels, isohaemagglutinin levels, ni-

troblue-tetrazolium test (NBT), human immunodeficiency virus (HIV) antibody, the titer of specific antibodies for tetanus, diphtheria or pneumococcal antigens, purified protein derivative (PPD) test, and the skin prick test. The inhalant allergens for the later test include trees (elm, maple, oak), grasses (Bermuda grass, Johnson grass, June grass [Kentucky bluegrass]), weeds (common ragweed, cocklebur, rough pigweed), molds, and miscellaneous allergens (mite, dog dander, cat dander, cockroach) (31). In addition to inhalant allergens, we evaluated allergens that patients mentioned.

Moreover, serum complement components' levels, sweat test, spirometry, flow-cytometry analysis, complementary imaging and pathological investigation were also performed under some special conditions, as explained before in the literature (6,14,32). The final diagnosis of patients was recorded as follow: healthy children, PID, secondary or acquired immunodeficiency (SID), anatomical or functional disorders, and allergic conditions (33-36). Patients with a definite diagnosis of PID were classified based on the final reports of the International Union of Immunological Societies (IUIS) (33).

Statistical analysis

Statistical analysis was performed, using a commercially available software package (SPSS Statistics 17.0.0, SPSS, and Chicago, Illinois). For the comparison of the age of onset and diagnosis between different groups, analysis of variance (ANOVA) was used, while for categorical variables the chi-square test was used. A p value of less than 0.05 was considered statistically significant in our study.

Results

General information

We included 100 patients with recurrent infections according to criteria explained in Materials and methods section. Among these patients, 67% were male and 33% were female. The mean age of patients was 6.2 ± 4.0 years. Although all patients had a chief complaint of recurrent infections, the patients were categorized into 6 main groups according to main affected organs. We found out that the most frequent complaints were lower respiratory infections (31%) and upper respiratory infections (30%), followed by severe systemic infections (13%), gastrointestinal infections (11%), soft tissue infections or abscess (11%) and skin infections (4%).

Consanguineous marriage rate was 38% overall and there were only 2 patients with a family history of PID. One patient was a 9-year old girl with a history of common variable immunodeficiency (CVID) in her maternal uncle; she had recurrent pneumonia due to her congenital heart disease and there was no serologic evidence of PID. The second patient was a 5-year old boy with hyper IgM syndrome (HIgM) with a history of death in his infant sibling and his maternal uncle, also diagnosed with the same syndrome.

Comparison of groups' characteristics

After careful laboratory tests and evaluation of clinical diagnostic criteria, we categorized patients with a history of recurrent infections into the following 5 subgroups: healthy children, PID, SID, anatomical or functional disorders and allergic conditions. Accordingly, we diagnosed 30 cases as healthy, 18 as allergy, 16 as SID, 15 as anatomical or functional disorders, and 21 as PID (**table I**). In PID group, the mean age of patients was 6.3 ± 4.6 years and there were 11 (52.3%) males in this group. Fifteen patients born of consanguineous marriages (71.4%, **figure 1**) were seen in the PID group. The mean age at time of the first manifestation was 4.6 ± 4.0 years and the mean diagnostic delay in this group was 2.8 ± 0.3 years. There was only 1 HIgM (4.5%) case with a family history of PID. The most frequent presentation in this group was soft tissue infections or abscess, in 7 (33.3%) patients.

The main diagnosis in allergy group was hyper-reactive airway disease (HRAD including small hyper-reactive airway) with the frequency of 15 (83%) of all 18 cases. The coincidence of HRAD and gastro-esophageal reflux disease (GERD) were seen in 1 (5.6%) case in allergy group, while the two remaining patients' diagnoses were asthma 2 (11%).

The main disease in anatomical or functional defects group was cystic fibrosis with a frequency of 5 (33.3%) cases, followed by congenital heart disease in 4 (26.7%), GERD in 2 (13.3%) and hypertrophic adenoids in 2 (13.3%). Interstitial lung disease and nasal septum deviation were each identified in one patient.

In the SID group, HIV infection in 4 (25%) and corticosteroid side-effect in 3 (19%) cases were the main evidenced causes of recurrent infections. Regarding the other SID diseases, there was 1 (6.3%) each with cytomegalovirus infection, Crohn's disease, protein-losing enteropathy, fatty acid oxidation disorder, Gaucher's disease, juvenile polyposis, intestinal lymphangiectasia, lymphoma, and maple syrup urine disease.

Demographic and clinical data of other causative groups of recurrent infection are summarized in **table I**. PID group presented a significantly higher rate of parental consanguinity (p = 0.001) and soft tissue infections (p = 0.004). In contrast, healthy individuals significantly manifested upper respiratory infection (p = 0.024), while the lower respiratory infection was more frequent in cases with anatomical or functional defects (p = 0.001). Sex of patients did not influence the incidence of PID or anatomical disease, but there was a slightly higher rate of males in allergy and SID.

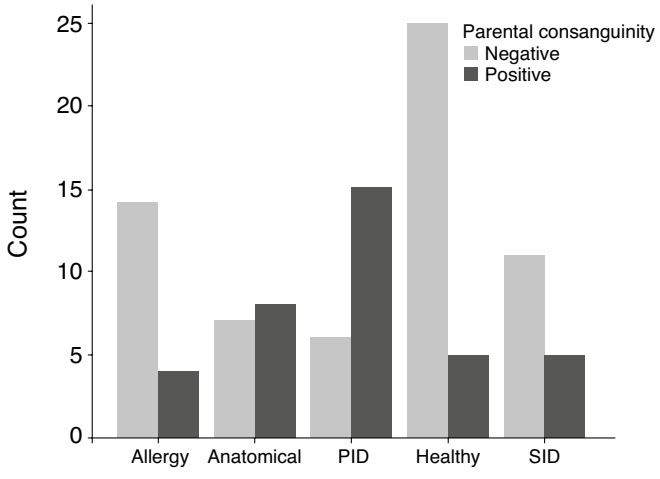
Table I *- Classification of demographic and clinical data of 100 pediatric individuals with complaint of recurrent infection based on the final definite diagnosis.*

Parameters	**total (n = 100)**	**healthy (n = 30)**	**atopy (n = 18)**	**anatomical (n = 15)**	**SID (n = 16)**	**PID (n = 21)**	***p*-value**
sex (m/f)	67/33	22/8	14/4	7/8	13/3	11/10	0.099
onset age, years (SD)	3.7 (3.0)	2.1 (2.0)	3.9 (1.8)	4.4 (4.8)	2.8 (1.4)	4.6 (4.0)	0.321
diagnosis / current age, years (SD)	6.2 (4.0)	6.0 (3.5)	5.8 (3.0)	7.8 (5.2)	5.1 (3.8)	6.3 (4.6)	0.482
consanguinity (%)	37 (37.0)	5 (16.6)	4 (22.2)	8 (53.3)	5 (31.2)	15 (71.4)[a]	0.001
positive family history (%)	2 (2.0)	0	0	1 (6.6)	0	1 (4.5)	0.52
episodes of infection / year (SD)[1]	5.9 (4.7)	5.3 (2.7)	6.9 (3.2)	4.8 (2.0)	7.4 (3.6)	6.6 (4.0)	0.23
Major complications							
upper respiratory infection (%)	30 (30.0)	14 (46.7)[c]	8 (44.4)	3 (20.0)	2 (12.5)	3 (14.3)	0.024
lower respiratory infection (%)	31 (31.0)	2 (6.6)[d]	8 (44.4)	10 (66.6)[d]	6 (37.5)	5 (23.8)	0.001
gastrointestinal infection (%)	11 (11.0)	3 (10.0)	1 (5.5)	2 (13.3)	4 (25.0)	1 (4.8)	0.320
skin infection (%)	4 (4.0)	1 (3.3)	0	0	0	3 (14.2)	0.097
soft tissue infection or abscess (%)	11 (11.0)	3 (10.0)	0	0	1 (6.2)	7 (33.3)[e]	0.004
severe infection (%)	13 (13.0)	7 (23.3)	1 (5.5)	0	3 (18.8)	2 (9.5)	0.156
Laboratory tests							
white blood count (cell/ul)	13230 ± 7120	15229 ± 4330	13107 ± 4591	16211 ± 3060	11028 ± 3416	7023 ± 6028	0.07
lymphocytosis (%)	72 (72.0)	28 (93.3)	14 (77.8)	15 (100)	5 (31.2)	10 (47.6)	<0.001
leukopenia (%)	9 (9.0)	0	0	0	3 (18.7)	6 (28.5)	0.001
neutrophils (cell/ul)	7450 ± 7277	7952 ± 3171	8341 ± 2400	8591 ± 2803	5801 ± 2974	3704 ± 3380	0.05
neutropenia (%)	5 (5.0)	0	0	0	1 (6.2)	4 (19.0)	0.01
lymphocytes (cell/ul)	5200 ± 5147	5427 ± 2730	7379 ± 4100	5278 ± 3003	5914 ± 5774	3710 ± 3099	0.03
lymphopenia (%)	9 (9.0)	0	0	0	4 (25.0)	5 (23.8)	0.002
high erythrocyte sedimentation rate (%)	86 (86.0)	25 (83.3)	17 (94.4)	15 (100)	8 (50.0)	21 (100)	< 0.001
high C-reactive protein (%)	85 (85.0)	26 (86.6)	17 (94.4)	14 (93.3)	7 (47.7)	21 (100)	< 0.001
human immunodeficiency virus (%)	4 (4.0)	0	0	0	4 (25.0)	0	< 0.001
low serum IgM level (%)	7 (7.0)	0	0	0	1 (6.2)	6 (28.5)	< 0.001
higher serum IgM level (%)	29 (29.0)	5 (16.6)	7 (38.8)	9 (60.0)	5 (31.2)	3 (14.2)	0.01
low serum IgG level (%)	5 (5.0)	0	0	0	1 (6.2)	4 (19.0)	0.01
higher serum IgG level (%)	27 (27.0)	7 (23.3)	7 (38.8)	3 (20.0)	7 (47.7)	3 (14.2)	0.21
low serum IgA level (%)	8 (8.0)	0	0	0	1 (6.2)	7 (33.3)	< 0.001
low isohaemagglutinin titers (%)	8 (8.0)	0	0	0	0	8 (38.0)	< 0.001

Parameters	total (n = 100)	healthy (n = 30)	atopy (n = 18)	anatomical (n = 15)	SID (n = 16)	PID (n = 21)	*p*-value
low anti-P23 pneumococcal antibody (%)	5 (5.0)	0	0	0	0	5 (23.8)	< 0.001
low anti-tetanus antibody (%)	5 (5.0)	0	0	0	0	5 (23.8)	< 0.001
low anti- diphtheria antibody (%)	7 (7.0)	0	0	0	0	7 (33.3)	< 0.001
low $CD3^+$ T-cells (%)	3 (8.0)	0	0	0	0	3 (14.2)	0.02
low $CD4^+$ T-cells (%)	5 (5.0)	0	0	0	0	5 (23.8)	< 0.001
low $CD8^+$ T-cells (%)	3 (3.0)	0	0	0	0	3 (14.2)	0.02
low $CD19^+$ B-cells (%)	4 (4.0)	0	0	0	0	4 (19.0)	0.003
low $CD16\text{-}56^+$ NK-cells (%)	3 (3.0)	0	0	0	0	3 (14.2)	0.02
negative nitroblue-tetrazolium test (%)	0	0	0	0	0	0	-
negative purified protein derivative test (%)	4 (4.0)	0	0	0	0	4 (19.0)	0.003
positive skin prick test (%)	19/26 (73.0)	NI	15/18 (83.3)	NI	NI	4/8 (50.0)	0.46
defective spirometry test-higher than 6 years (%)	16/48 (33.3)	0/11 (0)	7/7 (100)	2/9 (22.2)	2/8 (25.0)	5/13 (38.4)	0.06

Abbreviations: SID, secondary immunodeficiency; PID, primary immunodeficiency; not indicated.
[1]Including episodes of viral infections, common cold and flu.
[a]$p < 0.001$ and $p = 0.016$ in comparison with healthy and anatomical groups, respectively.
[c]$p = 0.016$ and $p = 0.02$ in comparison to PID and SID groups, respectively.
[d]$p < 0.00$ in comparison to healthy group.
[e]$p = 0.008$, $p = 0.042$ and $p = 0.045$ in comparison to allergy, anatomical and healthy groups.

Figure 1 *- Consanguinity frequency in different diagnostic groups of 100 pediatric individuals with complaint of recurrent infection. SID, secondary immunodeficiency; PID, primary immunodeficiency.*



Intragroup comparison of PID

According to IUIS classification, we classified PID cases as following: 3 patients of combined immunodeficiency (all with severe combined immunodeficiency), 2 of well-defined syndromes with immunodeficiency (both with ataxia-telangiectasia), 6 of predominantly antibody deficiencies (X-linked agammaglobulinemia in 33.3% and CVID, HIgM, IgA deficiency and undefined hypogammaglubulinemia each in 16.6%), 8 of congenital defects of phagocytosis (Mendelian susceptibility to mycobacterial disease and severe congenital neutropenia each in 37.5% and leukocyte adhesion deficiency in 25%), one of immune dysregulation with hemophagocytic lymphohistiocytosis disease, and one of defects in innate immunity with anhidrotic ectodermal dysplasia. There were no patients with a diagnosis of autoinflammatory disorders or complement deficiencies in the present survey.

In antibody deficiencies group, the mean age of patients was 7.3 ± 5.7 years and the mean age of onset was 6.2 ± 6.0 years, which had a later onset and diagnosis comparing to phagocytosis disorders (6.9 ± 4.8 and 4.0 ± 3.2 years, respectively) and combined

Table II *- Comparisons of main affected organ by infections in different types of PID patients.*

Parameters	PID (n = 21)	Combined immunodeficiency (n = 3)	Antibody deficiencies (n = 6)	Defects of phagocytosis (n = 8)	Other PIDs (n = 4)
upper respiratory infection (%)	3 (14.3)	-	1 (16.6)	-	2 (50)
lower respiratory infection (%)	5 (23.8)	1 (33.3)	4 (66.7)	-	-
gastrointestinal infection (%)	1 (4.8)	1 (33.3)	-	-	-
skin infection (%)	3 (14.2)	-	1 (16.6)	-	2 (50)
soft tissue infection or abscess (%)	7 (33.3)	-	-	7 (87.5)	-
severe infection (%)	2 (9.5)	1 (33.3)	-	1 (12.5)	-

immunodeficiencies (2.7 ± 0.57 and 2.5 ± 0.38 years, respectively), but these differences were not statistically significant (p = 0.37 and p = 0.17, respectively).

There were 7 (87.5%) females with phagocytosis disorders, but there was a male dominance in the antibody deficiency (100%) and in the combined immunodeficiency (67%) groups; in which phagocyte disorders were significantly more frequent among females than males compared to antibody deficiencies (p = 0.007). Parental consanguinity was recorded in the 87.5% of phagocyte disorders and 100% of combined immunodeficiency groups. In the antibody deficient patients, however, there were 3 (50%) consanguineous marriages (p = 0.124).

Regarding to the first presentation and the main organ involvement, among phagocytosis disorders there were 7 (87.5%) patients with soft tissue infections, in antibody deficiency group there were 4 (66%) patients with lower respiratory infections, and in combined immunodeficiencies group, there was 1 (33.3%) patient with a lower respiratory infection, 1 (33.3%) with a gastrointestinal infection and 1 (33.3%) with severe systemic infections (p = 0.015, **table II**). Comparing antibody deficiency and phagocyte groups, systemic and soft tissue infections were significantly more frequent in phagocyte group (p = 0.012), while respiratory tract infections were the main presentation of in antibody deficiency group (p = 0.04).

Discussion

A considerable proportion of children with recurrent infections referred to the tertiary hospitals may have PID, and parental consanguinity was significantly higher in this group, comparing to other causes of recurrent infections. Moreover, presenting symptoms of children with recurrent infections may be helpful to determine the underlying causes of disease, as recurrent soft tissue infections strongly suggest a PID especially in phagocytosis disorders, and recurrent lower respiratory tract infection suggest anatomical / functional defects or an antibody immunodeficiency.

In our previous retrospective study on the rate of PID among children with recurrent infections in 2012 in the same tertiary hospital, we have shown that only 11% of 260 patients were labeled as PID (6), whilst by applying the recommended stepwise guideline to these patients in the current prospective study, this rate was higher (21%), suggesting a risk of lower-estimation and undiagnosed mild form of PID among patients underwent non-systematic approach to recurrent infections.

Parental consanguinity seems to be a key finding in the PID group, as there was about 72% of consanguinity in the family history of these patients. Although the consanguinity was also frequent in patients with anatomical disorders (53.3%, in 5 patients with cystic fibrosis, 2 patients with congenital heart disease and 1 patient with interstitial lung disease) this rate was lower than PID patients. In the PID subgroups section, 90% of phagocyte disorders group, 50% of antibody deficiency group, and 100% of combined immunodeficiency group had consanguineous parents. In a previous study in 2013, the mean proportion of consanguineous marriages was 65.6% among Iranian PID patients who were registered in the database, while the overall rate was 38.6% in general population of Iran. However, the rate of consanguinity was reported about 76% in combined immunodeficiency, 73% in defects of phagocytic function, and 54% in predominantly antibody deficiencies (37). Although genetic analysis was not the scope of this study, the findings of higher consanguinity and slightly higher rate of male gender in antibody and combined immunodeficiencies reflect the presence of autosomal recessive and X-linked inheritance pattern of PID in our cohort, respectively, as has been reported previously (38,39). These findings are in accordance with other reports from the Middle Eastern region (40) with a high rate of consanguineous marriage, and North of Africa countries (41,42). No patient with a final diagnosis of complement deficiencies or autoinflammatory diseases was recorded in this survey. As a fact, among PIDs, complement deficiencies are relatively rare

Table III *- Comparisons of median delay in diagnosis of different types of PID patients diagnosed with and without systematic approach to recurrent infection.*

Cause of recurrent infections	Patients diagnosed without systematic approach to recurrent infection, years (range)	Patients diagnosed with systematic approach to recurrent infection, years (range)	p-value
primary immunodeficiency	4 (0-33)	2.8 (0-5)	0.003
combined immunodeficiency	0.16 (0-12)	0.13 (0-1.2)	0.07
antibody deficiencies	2.13 (0-28)	1.3 (1-5)	0.01
defects of phagocytosis	0.5 (0-15)	0.2 (0.1-1.7)	0.04

(less than 2% of total PID registry in Iran [37]), and the majority of patients with complement deficiencies do not present with increased susceptibility to infections, and usually suffer from hereditary angioedema and autoimmune disorders such as systemic lupus erythematosus, glomerulonephritis, vasculitis and autoimmune cytopenia (43,44). Of course, this depends on which complements are missing, but terminal complement deficiencies presenting with recurrent *Neisseria* infections including meningitis, are very rare disorders.

Similarly, patients who have an autoinflammatory disease such as familial Mediterranean fever rarely complain from recurrent or chronic infections (45,46). Therefore, it would be noted that non-infectious warning signs of PID particularly associated with complement deficiencies and autoinflammatory diseases, should be combined in the current guideline of approach to recurrent infectious patients.

Moreover, the findings of this survey demonstrated that attention to presenting symptoms of children with recurrent infections may be helpful to target complementary para-clinical tests, and to determine the underlying causes of disease. Based on our findings, recurrent soft tissue infections strongly suggest a PID, especially in phagocytosis disorders. In contrast, recurrent upper respiratory tracts infections usually presented as a mild condition in healthy individuals, and lower respiratory tract infection presented in those patients with anatomical or functional defects.

It should be noted that this data was collected in a tertiary referral hospital; therefore, most of our patients in this study were referred from other primary / peripheral centers. Indeed, some of them had many prior admissions or visits with a chief complaint of recurrent infections; so, a higher incidence of PID would be expected than in general populations. In addition, the higher percentage of SID or anatomical disease and a lower rate of healthy patients in this study might reflect this notion.

Although the findings of unusual pathogens have been reported to be crucial for diagnosis of PID (6), infection due to enhanced susceptibility to disease with a specific common germs even in a single organ can however be associated with PID (47). Moreover, most of the patients with recurrent infections were under different antibiotic therapies, which directly affect the microbiologic evaluation (48). Therefore, we did not aim to evaluate the pathogens of patients in this study.

One of the important results of this survey was the evidence of reduction of PID diagnostic delay through a stepwise approach that we previously elucidated (6). Based on that approach, in the present study we evaluated the distribution of recurrent infections among 100 patients. The mean diagnostic delay of PID in this study was 2.8 years, which was significantly lower than 4 years, indicated in our previously published registry report (37). **Table III** demonstrates the delay in diagnosis in each main PID disorder, in which antibody deficiency and phagocytosis showed significant lower delay in diagnosis prospectively compared to previously published patients (6). Intriguingly, the rate of long-term complication of these earlier discovered patients decreased significantly (particularly bronchiectasis in group of antibody deficiency decreased from 25% to 16.6%), and the chance of performing hematopoietic stem cell transplantation were increased (less than 5% to 66.6% in group of combined immunodeficiency), suggesting the importance of timely diagnosis by the established guideline on treatment and management of PID patients.

However, there is a meaningful lag to timely diagnosis and treatment of PID, even after hospital admissions as observed in the medical history of currently studied patients. This fact explains the necessity of attention to increasing awareness of physicians and the need for proper laboratory tests in peripheral centers other than referral hospitals (21). In a developing country like Iran, there is insufficient medical equipment, and also an unequal distribution of diagnostic facilities. For example, NBT is a common test for diagnosis of CGD in Iran, and the current diagnostic criteria were based on the result of this method. NBT

is an old test that is not considered the currently preferred methodology for the diagnosis of CGD, and we hope change NBT to flow cytometric method as a common test for diagnosis of CGD in future in Iran.

Regular and continuous education should be considered for pediatricians and general practitioners to inform them about updated screening steps and preliminary diagnostic tests to perform timely referral to a specialist when a chronic condition such as PID is suspected.

Conflict of interest

The authors declare that they have no conflict of interest.

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Anaphylaxis in a food allergy outpatient department: one-year review

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Key words
adults; anaphylaxis; cofactors; food allergy; pediatric

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Doi
10.23822/EurAnnACI.1764-1489.45

Summary
Background. *Anaphylaxis is an acute, potentially fatal, multi-organ allergic reaction. Our aim was to characterize the population with food induced anaphylaxis followed over a one-year period.* ***Methods.*** *Retrospective analysis of clinical files of patients with food anaphylaxis observed in our food allergy consultation during 2016.* ***Results.*** *Sixty-two patients were included. In the pediatric group, the implicated allergens were cow's milk, egg and fish and in the adults' group, the commonest allergens were nuts and wheat. Allergy to shrimp affected equally children and adults. The most frequent symptoms were urticaria (85.5%), angioedema (64.5%) and dyspnea (62.9%). Cofactors were present in 32.6% of patients, mainly exercise. Asthma and/or rhinitis were the most frequent comorbidities.* ***Conclusion.*** *In accordance to other studies, milk and egg were the most implicated allergens in children. Anaphylaxis in adults reflects the Mediterranean sensitization pattern. Exercise was the most relevant cofactor.*

Introduction

Food allergy is an adverse reaction to foods in which immunologic mechanisms have been demonstrated (1), whether IgE-mediated, non-IgE-mediated, or involving a combination of IgE- and non-IgE-mediated mechanisms. It can result in life threatening reactions and has a significant impact on quality of life (2). In the last decades, the prevalence of food allergies has increased in several regions throughout the world (3). Although food allergy is not as prevalent as other allergic diseases, its repercussions on dietary habits and social interaction is quite relevant (4). Allergic reactions to foods are the leading cause of anaphylaxis in patients of all ages outside the hospital setting (5); however, the precise risk of anaphylaxis is unknown (2). Recent data shows an increase in emergency department visits and admissions for food-induced anaphylaxis (5). Although all foods, theoretically, can induce anaphylaxis, only a restricted number of foods are responsible for the majority of the reactions (3).

Because the severity of allergic reactions to foods cannot be predicted either by the severity of prior reactions or by allergy test results, appropriate recognition of signs and symptoms and prompt initiation of treatment are necessary for optimal outcomes (5).

Incomplete recognition of the signs and symptoms of anaphylaxis can be life-threatening to the patient (5). Allergy testing is quite relevant to determine the cause of anaphylaxis and therefore prevent further reactions. Specialized counseling in a food allergy outpatient is very important, and can be highly efficient. It is important to identify which allergen is responsible for the reaction, and this investigation can include skin testing, specific IgE (sIgE) determination and/or oral food challenges (OFC)

(3,6). The identification of molecular allergens involved can help determine the severity of reaction and relevant cross-reactivities (7).

Food allergy prevalence varies considerably between studies, but is estimated to affect up to 10% of children and 2-3% of adults (2). One European systematic review on the epidemiology of anaphylaxis lists foods as one of the most common causes (8). In Portugal, Gaspar et al, in their study on the frequency of pediatric anaphylaxis, obtained an 84% prevalence of food-induced anaphylaxis (9). Asero et al, in 1100 food-allergic patients, obtained a 5% incidence of food-related anaphylaxis in patients diagnosed with food allergy, over a one year period (10). Two Australian cohorts studies performed in 2001/2002 and 2005/2006, reported seafood, fish and peanut as the most frequently accountable food groups (11). In children, peanuts and tree nuts were the most frequently identified in several studies, but milk, egg and shrimp were also commonly documented (12).

Admission rates for anaphylaxis differ between countries (11). In the USA, food induced anaphylaxis is the leading cause of anaphylaxis treated in emergency departments (ED) (13). Moreover, an upward trend was noticed in the United Kingdom, Italy and New Zealand (11,13).

It is unclear why the highest rates of food-anaphylaxis predominate in children under 5 years of age. Possible reasons include high prevalence of food allergy in this age group, and the possibility that severe reactions are more common before a correct diagnosis is made (2).

Fatalities are more commonly seen in young adults (14), but have a very low incidence rate in preschool years (2). Most importantly, 30% of fatal anaphylaxis cases are triggered by food allergens (5).

The aim of this study is to characterize the population with food induced anaphylaxis followed in our food allergy outpatient department over a one-year period.

Methods

Medical record review

We reviewed the medical records of all patients evaluated in the Food Allergy outpatient of the Allergy and Clinical Immunology Department at Coimbra University Hospital, from January to December 2016. Those with a diagnosis compatible with anaphylaxis in any period of their lives, were selected to be part of the study.

Patients of all age groups were included, and were categorized into clusters according to age and culprit food for the first anaphylactic episode. Although some of them later displayed allergic symptoms with other foods, these culprits were excluded from this study.

The clusters were: cow's milk, egg, meat, fish, shellfish, wheat, fresh fruits, tree nuts, peanut and mushroom.

Since the study was retrospective, and based on routine investigations performed on patients spontaneously presenting at the hospital for suspected food allergy, no institutional ethical permission was needed.

Data collection

Physician investigators reviewed 358 medical records of food-allergic patients followed in the food allergy outpatient department (FAOD) of Coimbra's University Hospital.

Exclusion criteria included absence of anaphylaxis clinical criteria, missing data (records lacking documentation of signs and symptoms of anaphylaxis) or loss of follow-up.

All patients with symptoms suggestive of food anaphylaxis were further investigated with skin and/or in vitro testing. Oral food challenges (OFC) to confirm allergy or tolerance were performed in selected cases.

Results were gathered to evaluate demographic data, culprit food, presenting symptoms, treatment options and the presence of atopy. Date of the first anaphylactic event, reported by the patient, was documented.

Anaphylaxis was defined according to the Anaphylaxis guidelines from the European Academy of Allergy and Clinical Immunology (EAACI) (15).

Atopy was defined according to the World Allergy Organization (WAO) (16) and EAACI (12) guidelines. Atopic comorbidities were evaluated and registered in our study population.

Cofactors such as exercise, intake of alcohol and drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) and angiotensin converting enzyme inhibitors (ACEI) were documented, and were defined according to the EAACI guidelines (1).

Statistical analysis

The results are presented in absolute and relative frequencies. Quantitative variables with normal distribution are expressed as mean ± standard deviation. Variables not normally distributed are expressed as median (IQR).

Results

From a total of 358 patients observed in the food allergy outpatient department during 2016, 62 patients (17.3%) met criteria for anaphylaxis. Demographic data are summarized in **table I**.

Culprit foods involved in anaphylaxis

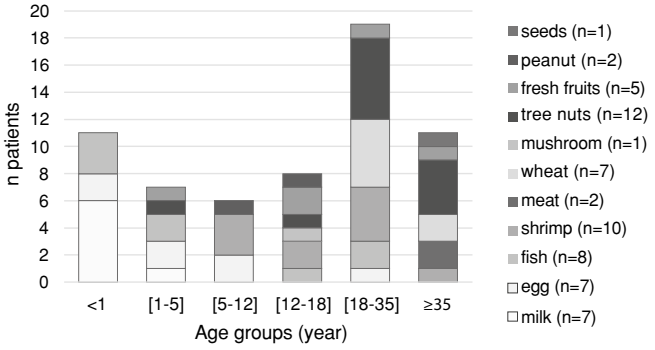
The foods implicated in anaphylaxis according to the age of the first episode are specified in **figure 1**. Tree nuts (n = 12), shrimp

Table I *- Demographic characteristics of the study participants.*

Characteristic	Children n = 32 (51.6%)	Adults n = 30 (48.4%)	No. of patients n = 62
Male	18 (56.3%)	17 (56.7%)	35 (56.5%)
Atopic comorbidities	25 (78%)	22 (73%)	47 (75.8%)
AA	1 (3.1%)	4 (13.3%)	5 (8.1%)
AR	2 (6.2%)	11 (36.7%)	13 (21%)
AA + AR + AD	5 (15.6%)	1 (3.3%)	6 (9.7%)
AA + AR	12 (37.5%)	6 (20%)	18 (29%)
AR + AD	5 (15.6%)	0	5 (8.1%)

Abbreviations: AA, allergic asthma; AR, allergic rhinitis; AD, atopic dermatitis.

Figure 1 *- Causes of food-induced anaphylaxis according to the age of the first reaction.*



(n = 10) and fish (n = 8) were the most common causes of anaphylaxis in our population. **Table II** summarizes the prevalence of all culprit foods and **table III** showcases demographic and clinical data for the most common causative foods.

Important differences exist in the presentation and etiology of food anaphylaxis between adults and children. Therefore, we analyzed the two populations separately.

Pediatric population

Cow's milk was the most frequent cause of food anaphylaxis in this population (n = 7), and the highest incidence in children below 1 year of age (n = 5). The earliest report of anaphylaxis to milk was in a 15-day newborn boy.

Table II *- Triggers of anaphylaxis.*

Cause	No. (%) of patients (n = 62)	No. (%) of adults (n = 30)	No. (%) of children (n = 32)
tree nuts (walnut 4, hazelnut 4, almond 1, chestnut 1, pistachio 1, cashew 1)	12 (19.4%)	10 (33.3%)	2 (6.3%)
shrimp (n = 10)	10 (16.1%)	5 (16.7%)	5 (15.6%)
fish (codfish 3, salmon 3, whitefish 1, redfish 1)	8 (12.9%)	2 (6.7%)	6 (18.8%)
milk (cow's milk 7)	7 (11.3%)	0	7 (21.9%)
egg (whole egg 4, raw egg white 1, cooked egg white 1, Bird-egg syndrome 1)	7 (11.3%)	1 (3.3%)	6 (18.8%)
wheat (n = 7)	7 (11.3%)	7 (23.3%)	0
fresh fruits (peach 3, apple 1, kiwi 1)	5 (8.1%)	2 (6.7%)	3 (9.4%)
meat (pork 2)	2 (3.2%)	2 (6.7%)	0
peanut (n = 2)	2 (3.2%)	0	2 (6.3%)
mushroom (n = 1)	1 (1.6%)	0	1 (3.1%)
seeds (sunflower seeds 1)	1 (1.6%)	1 (3.3%)	0

Table III - *Culprit foods: comparison by the most common food causes. (IQR - Interquartile range).*

Cause	Age, median (IQR), y	Total IgE median (± SD), kU/L	No. of patients (%) with atopic comorbidities
Total	21.3 (0.08 - 66.0)	304 (± 446.7)	47 (75.8%)
tree nuts	32.2 (2.0 - 62.0)	389.5 (± 278.1)	11 (17.7%)
shrimp	24.4 (6 - 66)	602 (± 553.9)	10 (16.1%)
fish	9.1 (0.48 - 30)	646.5 (± 533.2)	5 (8%)
milk	1.6 (0 - 10)	219 (± 232.1)	6 (9.7%)
egg	6.4 (0.72 - 20)	638 (± 567.9)	6 (9.7%)
wheat	37.8 (20 - 64)	404.9 (± 263)	3 (4.8%)

Six patients (85.7%) had no previous diagnosis of allergy to cow's milk protein (CMP) before the anaphylactic episode. Of those, the anaphylactic reaction occurred in 4 children after the first intake of adapted milk formula (between 15 days and 2 months of age), all with previous exclusive breastfeeding, and in 2 patients after the intake of puree containing milk, both at 6 month-old.

Only one patient had a first episode of anaphylaxis to milk occurring after the first year of life. This 10 year-old boy had a known diagnosis of allergy to CMP, and the episode of anaphylaxis occurred after an accidental ingestion of food with trace amounts of milk. He later developed symptoms of allergy to shrimp (oral allergy syndrome and urticaria) in adulthood, but never had an episode of anaphylaxis to this food. Another patient allergic to milk also presented allergic symptoms with the ingestion of cow's meat and fruits from the *Rosacea* family. This patient was sensitized to Bos d 6, but not to *Rosacea* fruits lipid transfer protein (LTP). Four patients (57.1%) underwent an OFC with milk, all positive. Three patients had an immediate reaction in the OFC but one had a late reaction (abdominal cramps and diarrhea). Of those, only one patient underwent an oral tolerance induction protocol (at 15 years old) which drastically diminished the number of accidental reactions. All the patients kept follow-up until adulthood, and none of them acquired natural tolerance to milk.

Among the 6 children with anaphylaxis to egg, three were already known to have egg allergy before the anaphylactic episode. All of these patients had egg allergy symptoms since the first year of life, and the anaphylactic episode occurred with food containing egg as an occult allergen. The anaphylaxis was the first symptom of egg allergy in two patients (33.3%), and were elicited by cooked egg yolk on the first attempt to introduce egg in the diet. All reactions were IgE-mediated, with all patients having positive specific IgE (sIgE) to egg yolk, egg white, ovomucoid (Gal d 1) and ovalbumin (Gal d 2).

Of the 6 patients with anaphylaxis to egg, 3 are in absolute egg avoidance, 2 are in avoidance of raw egg and 1 tolerates extensively cooked egg. None of the patients underwent tolerance induction protocols, due to the severity of the egg allergy.

Five patients (83.3%) had atopic comorbidities.

The third most frequent cause of anaphylaxis in our pediatric population was fish, in 6 patients (18.8%). In the pediatric population, all the anaphylaxis to fish occurred in the first year of life with the introduction of this food in the infant diet. Codfish was the most common cause (60%), but red fish and salmon were also implicated. All reactions were IgE mediated, with positive skin prick tests (SPT) and sIgE. OFC with alternative fish were performed in all patients, and two challenges were positive (codfish and mackerel). During follow-up, 2 patients are in strict avoidance of all fishes, 2 patients only tolerate tuna, and 2 patients acquired tolerance to fish and now have no food restrictions.

The episodes triggered by shrimp (n = 5) were more frequent in school-age children and adolescents (6 to 14 years). All reactions were IgE mediated, and all patients presented sensitization to multiple shellfish. Eighty percent of the patients (n = 4) were sensitized to shrimp tropomyosin, Pen a 1. Four patients presented symptoms of rhinitis related with house dust mite exposure and had positive specific IgE to mite tropomyosin, Der p 10. All the patients are in strict avoidance of shellfish.

Peanut was the culprit food in only two patients. Both patients had symptoms with the ingestion of tree nuts and fresh fruits and were sensitized to peach LTP, Pru p 3. None of them tolerates peanut.

The anaphylaxis due to kiwi was in a 4 year-old boy; he had positive prick-test and sIgE to kiwi, but none of the kiwi's molecular components were searched. This patient also presented positive sIgE to peach LTP and profilin, despite he never had complaints with the ingestion of any *Rosacea* fruits.

The patient with anaphylaxis due to mushroom was a 17 year-old boy who developed symptoms during a handball game in

hot and humid environment, after a meal containing meat and mushrooms cooked with wine. He had a personal history of allergic rhinitis. The patient had positive prick-to-prick test to *Agaricus bisporus* (the mushroom ingested), and positive prick test and sIgE to *Alternaria* alternata fungus. The cross-reactivity between *A. bisporus* and *A. alternata* was confirmed by SDS-PAGE Immunoblotting and Immunoblotting-inhibition assays. The patient underwent sublingual immunotherapy with *A. alternata* extract for 5 years which alleviated the allergic rhinitis symptoms, and he never had other systemic reaction to foods. Nowadays, the patient is in mushroom restriction.

Adult population

In our sample, anaphylaxis due to tree nuts was the most frequent cause of food anaphylaxis in the adult population (n = 10), and its frequency was higher in young adults (18 to 35 years). Of those patients, two had symptoms with the ingestion of fresh fruits and were sensitized to Pru p 3; two were sensitized to both Pru p 3 and wheat LTP, Tri a 14. One of the patients sensitized to both Pru p 3 and Tri a 4 had symptoms related to multiple cofactor-mediated food reactions to different groups of foods.

The second most frequent cause of anaphylaxis in the adult population was wheat; it was the culprit food in 7 patients (23.3%). All the reactions were wheat-dependent exercise induced anaphylaxis (WDEIA), and all patients tolerated wheat when eaten without exercise. In three patients, non-steroidal anti-inflammatory drugs (NSAIDs) were also cofactors. All patients presented sensitization to other flours namely: maize (n = 6), rye (n = 5) and barley (n = 3). Four patients (57%) were sensitized to ω-5-gliadin and 2 (28.6%) to wheat LTP, Tri a 14. Only 2 patients were atopic. None of the patients had symptoms with other foods.

Shrimp was the culprit food in five (16.7%) patients. Sixty percent of patients with allergy to shrimp were sensitized to Pen a 1. Only one patient had a cofactor-mediated reaction and it was due to the intake of ACEI drugs and alcohol. All patients were atopic.

One case of anaphylaxis due to egg was registered in a 20 year-old woman, with history of bird-egg syndrome. The anaphylaxis was the first manifestation of egg allergy.

Two patients had anaphylaxis to meat (a male and a female), and both were sensitized to galactose-alpha-1,3-galactose (alpha-gal). In regards to risk factors to meat allergy, both of them denied ever being bitten by thicks, but the male spent his childhood in Africa and the female was a farmer.

Anaphylaxis due to fish (n = 2), fresh fruits (n = 2) and sunflower seeds (n = 1) were also reported in the adult population.

Tables IV and **V** summarize the molecular components identified in the pediatric and adult population, and respective sIgE results.

Table IV *- Pediatric population: molecular components identified and respective mean sIgE.*

Molecular component	No. children sensitized	sIgE Mean (± SD), kU/L
Bos d 4	7	7.03 (± 12.9)
Bos d 5	7	9.53 (± 8.9)
Gal d 1	6	5.09 (± 12.1)
Gal d 2	6	8.88 (± 7.97)
Pen a 1	4	15.06 (± 34.7)
Gad c 1	4	2.09 (± 2.3)
Pru p 3	2	0.67 (± 1.9)

Table V *- Adult population: molecular components identified and respective mean sIgE.*

Molecular component	No. adults sensitized	sIgE Mean (± SD), kU/L
Pru p 3	4	29.15 (± 33.7)
Tri a 19	4	7.74 (± 9.02)
Tri a 14	4	10.05 (± 8.28)
Pen a 1	3	6.22 (± 55.23)
Gad c 1	2	0.63 (± 0.22)
Galactose-alpha-1,3-galactose (alpha-gal)	2	4.34 (± 1.17)

Signs and symptoms of anaphylaxis

Of the 62 patients who experienced anaphylaxis, 87.1% and 70.1% reported urticaria and angioedema, respectively. Shortness of breath (66.1%) was the second most frequent complaint, followed by laryngeal involvement (45.2%). **Table VI** summarizes the frequency of signs and symptoms of anaphylaxis according to the age group.

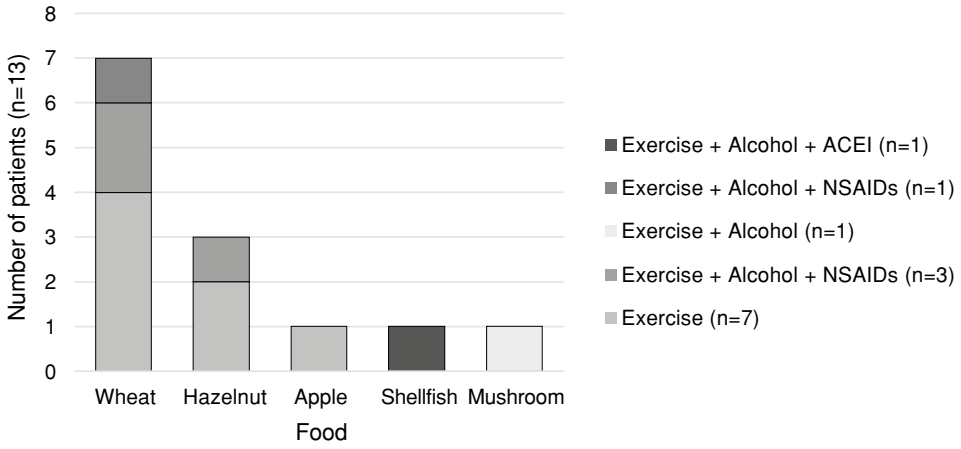
Emergency department attendance and treatment

Fifty-five patients (88.7%) resorted to the ED due to anaphylaxis symptoms. However, of the patients observed in the ED, only 18 (29%) were treated with epinephrine. From these, 15 (83%) had cardiovascular events, glottic edema or syncope / presyncope. The anaphylactic episodes related to the ingestion of tree nuts (n = 12) were the ones where epinephrine was more frequently administered (n = 5), followed by wheat-dependent

Table VI *- Signs and symptoms of associated to anaphylaxis.*

Sign or symptom	No. (%) of children (n = 32)	No. (%) of adults (n = 30)	Total (%) of patients (n = 62)
urticaria	27 (84.4%)	27 (90%)	54 (87.1%)
angioedema	21 (65.6%)	23 (76.7%)	44 (70.1%)
shortness of breath	20 (62.5%)	21 (70%)	41 (66.1%)
laryngeal involvement	9 (28.1%)	19 (63.3%)	28 (45.2%)
vomiting	16 (53.3%)	1 (3.3%)	20 (32.3%)
oral pruritus	8 (25%)	4 (13.3%)	12 (19.6%)
rhino-conjunctivitis	3 (9.4%)	7 (23.3%)	10 (16.1%)
syncope or presyncope	2 (6.3%)	6 (20%)	8 (12.9%)
hypotension	3 (9.4%)	6 (20%)	9 (14.5%)
diarrhea and/or abdominal cramps	1 (3.1%)	2 (6.7%)	3 (4.8%)
dysphagia	0	1 (3.3%)	1 (1.6%)

Figure 2 *- Cofactor mediated reactions according to culprit food.*



exercise induced anaphylaxis (n = 4) and shrimp-related anaphylaxis (n = 4). There were no differences in the use of epinephrine in patients with or without asthma.

Acute treatment of anaphylaxis also included antihistamines (58%), corticosteroids (47%), and bronchodilators (3%).

Of the patients observed in the ED (n = 55), only one had measurement of serum tryptase during the episode.

Cofactors and anaphylaxis

Thirteen patients (21%) had cofactor-mediated anaphylaxis. Cofactor mediated reactions according to culprit food are shown in **figure 2**.

Exercise was the most frequently implied cofactor followed by alcohol. Six patients had more than one cofactor, being exercise and alcohol the most common combination.

All anaphylaxis associated to wheat were exercise induced. Hazelnut was the second most frequent cause of food dependent exercise induced anaphylaxis (FDEIA).

Most of the FDEIA occurred in adults (n = 11); there were only 2 cases in the pediatric population, 2 male adolescents with exercise mediated reactions to apple and mushroom.

Discussion

The studies on the incidence and characteristics of food-induced anaphylaxis in both children and adults are few. There are some

studies that focus on the incidence of all causes of anaphylaxis in the Portuguese population (9,17,18), most of them concentrate on the pediatric population. Regarding the prevalence of food allergy in the Portuguese population only a handful of studies are available, and report prevalences of about 1% in the adult population (1) and 8.5% in the paediatric population (14). A study on self-reported food allergies, via telephone call, in a small sample of adults from the city of Oporto obtained a prevalence of 5.2% (12).

In an Italian study that analysed the prevalence of adult food anaphylaxis in 19 allergy outpatient clinics, they obtained a prevalence of 5% in a one-year period (10). A study that evaluated the incidence of anaphylaxis in a pediatric emergency department in Madrid cited that food was the most frequently suspected trigger of anaphylaxis, with a prevalence of 68% of children admitted with anaphylaxis (19). In a Boston cohort study with 1115 pediatric patients on the prevalence of food-related acute allergic reactions, authors concluded that in a 6-year period the annual number of visits for food-related acute allergic reactions increased from 164 to 391, with approximately half of these cases with criteria for food-induced anaphylaxis (20).

Perhaps due to the higher incidence of food allergy and, consequently, food anaphylaxis in children, there are more studies on the prevalence and characteristics of food anaphylaxis in this age group than in adults.

The prevalence of food anaphylaxis in our sample was 17.3%, which is higher than in most of previous studies. This could be due to the fact that our patients were taken from a consultation where most of the patients already had a diagnosis of food allergy, which can be a bias in our study. However, the prevalence of anaphylaxis can vary according to geographical and cultural factors.

The male preponderance (56.5%) in food-induced anaphylaxis presentations noted in this study is not consistent with other published studies that cited a slightly higher incidence in females (8,9,10,21).

So far, most studies in this field were done in English-speaking countries with dietary habits that are different from the Portuguese population.

In the pediatric population, the most common causes of anaphylaxis were milk (21.9%), egg (18.8%) and fish (18.8%). When compared with the results from English or American studies our results are quite different, as in English-speaking countries the most frequent causes are peanut, tree nuts, fish and milk (12,13,14,22). However, when compared with studies in European countries, especially in Mediterranean countries like Italy and Spain, the results are quite similar (milk, egg and tree nuts). This could be due to cultural differences, as peanut and peanut-derived products (e.g. peanut butter) is scarcely consumed in Portugal, whereas fish is a widely consumed food by the Portuguese population, and is introduced into the infant diet usually before 9 months of age, even when there is an atopic risk. The fact that roasted peanuts are more consumed in Portugal than peanut butter may contribute to the lower incidence of peanut allergy. There are some studies showing that the way peanuts are processed may profoundly influence the sensitization process (23). The high prevalence of sensitization to cow's milk and eggs, even before their introduction into the infant diet, could be owed to a possible sensitization during fetal life or after birth, through breast milk (24). Peanut anaphylaxis was listed in 2 patients (6.3%).

Among the adult population, tree nuts (33.3%), wheat (23.3%) and shrimp (16.7%) were the more commonly implicated foods. These results are more similar to those found in English-speaking countries, where reactions due to tree nuts, fish and shellfish are the most prevalent (20). Nevertheless, as in children, our results are comparable to those of the Mediterranean countries, where reactions due to LTP sensitization are frequent (10). Twenty percent of anaphylaxis occurred in patients sensitized to LTP.

All cases of wheat anaphylaxis were WDEIA. Of those, most patients (57.1%) were sensitized to ω-5-gliadin, which is coherent with previous studies that described this protein as the major allergen of immediate wheat allergy and WDEIA (7,23).

As previously reported by studies of food allergy prevalence in European countries, in our patients the types of foods most frequently implicated are included in the so-called "big eight allergens": milk, egg, peanut, tree nuts, wheat, soy, fish and shellfish (4). However, and as Lozoya-Ibáñez et al reported in their work about self-reported food allergy, the individual prevalence of each food type is distinct, which may be due to cultural differences in food habits. As in their study, our results may be due to the lower size of our sample in comparison with the other studies in this area.

Cutaneous (urticarial and/or angioedema) symptoms were the most common clinical manifestations, as has been previously described (4), followed by respiratory tract symptoms and gastrointestinal symptoms (19).

The mainstay of treatment of any kind of anaphylactic reaction is the timely administration of epinephrine. Epinephrine was administered in 29% of the patients, which is consistent with previous reports, describing a 25-44% administration rate (13). Tree nuts and wheat were the most common triggers in the epinephrine treated reactions; and we found no difference in the administration in patients with or without asthma.

Other therapies including H1-antihistamine, corticosteroids, bronchodilators, oxygen and fluid support are considered second- and third-line therapies (10). Systemic antihistamines are commonly used in anaphylaxis but have only been demonstrated to relieve cutaneous symptoms (10). Corticosteroids are not ef-

fective for the treatment of acute anaphylaxis, but may have a role in preventing or ameliorating biphasic or protracted anaphylaxis, which may occur in up to 20% of anaphylactic episodes (5).
Although these therapeutics ameliorate the symptoms of anaphylaxis, their use alone is not recommended in the treatment of the reaction (10).

Conclusions

Anaphylaxis is still an under-recognized and under-treated disease. Food anaphylaxis is a medical emergency which occurs unpredictably. It causes distressing symptoms to the individual, family and caregivers due to its potential to cause a fatal reaction. Unfortunately, in many situations epinephrine is not administered as the first line therapy, which can be dramatic in a severe episode.
Although allergy tests results correlate with the risk of reactivity to foods, they do not correlate with the severity of reactions. Thus, all physicians should be aware that any patient with food allergy is at risk of anaphylaxis at some point in their lives, and should inform them of the risks of their disease and how to deal with a severe reaction.
Our study is a contribution to the study of food allergies and anaphylaxis in Portugal and may be useful in comparison with other studies carried out in other countries.

Conflict of interest

The authors declare that they have no conflict of interest.

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Piperacillin-tazobactam anaphylaxis: a rare cause of occupational disease

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Key words

anaphylaxis; beta-lactams; drug hypersensitivity; occupational diseases; piperacillin

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Doi
10.23822/EurAnnACI.1764-1489.29

Summary

Piperacillin is a beta-lactam antibiotic of penicillin family. Some penicillins were reported as occupational diseases cause, but piperacillin anaphylaxis with occupational sensitization is rare. We describe the case of a female nurse with recurrent anaphylaxis in last few months without apparent cause, only in work environment. Latex allergy was excluded after negative latex glove provocation. Later during diagnostic workup, the patient reported a similar reaction minutes after piperacillin preparation. She denied any previous antibiotic therapeutic exposure. Skin prick tests (SPT) to beta-lactams were positive to piperacillin, penicillin G and major and minor determinants. SPT to cefuroxime was negative but intradermic test was positive. The patient has indication for beta-lactams eviction and for adrenaline auto-injector kit. No further reactions occurred after patient's transfer to another department with minimum possible exposure. Allergic risk prevention is essential and must be rapidly implemented to avoid incapacitating occupational diseases development.

Introduction

Anaphylaxis is a rapid-onset, multisystem hypersensitivity reaction with potentially fatal outcome (1). Clinically, anaphylaxis most frequent manifestations are cutaneous; however, respiratory, cardiovascular, gastrointestinal, and other symptoms may also occur (1). Drug-induced anaphylaxis (DIA) hypersensitivity mechanism is mainly an IgE-mediated response, but others have been characterized (1). Penicillin was in the past DIA most frequent cause, but was recently surpassed by amoxicillin (1). Healthcare professionals (HCP) are exposed to a large number of substances that act as allergens and/or irritants (2). These allergenic substances were known to cause contact dermatitis, but nowadays a wide spectrum of clinical manifestations like asthma, rhinitis, conjunctivitis and anaphylaxis is also included (2).

Piperacillin is an extended-spectrum beta-lactam antibiotic of the ureidopenicillin family, commonly used in combination with tazobactam, a beta-lactamase inhibitor. Some penicillins have been reported to cause occupational diseases (3-7), but only one case of piperacillin anaphylaxis with occupational sensitization has been described, and the diagnosis was only supported by serum IgE antibody detection (8). The authors describe the first case report of piperacillin anaphylaxis with occupational sensitization and diagnosis confirmed by skin tests.

Case report

A 28 year-old female nurse, with previous rhinitis history, was referenced to our outpatient clinic due to, in the last few months, recurrent episodes of generalized pruritus and cuta-

neous erythema, face swelling, chest urticarial papules, cough, dyspnea, wheezing and sometimes abdominal pain without apparent cause. The patient worked in the internal medicine ward for 5 years, and episodes were only work-environment related, excluding similar home episodes. These clinical manifestations usually resolved minutes after hydrocortisone intravenous administration. As other allergic diseases, the patient reported hand contact dermatitis with latex gloves. Patch testing previously performed in the dermatology department found a methylchloroisothiazolinone sensitization. The patient used only nitrile gloves, although latex gloves were used in the ward. In the first appointment the patient denied any association between the manifestations and food, drugs or latex exposure. She also denied previous surgeries, food or drug allergy and any previous antibiotic therapeutic exposure. Her parents also confirmed this last fact. Skin prick tests (SPT) identified sensitization to aeroallergens. Due to patient's occupation and work-environment involvement, a detailed latex allergy investigation was performed, including a latex glove provocation procedure that was negative. Due to diagnosis absence, the patient was instructed to register all possible triggers, and an adrenaline auto-injector kit was prescribed. Two months later, a similar reaction occurred minutes after piperacillin-tazobactam preparation in work context. The patient reconfirmed that she was never treated with any antibiotic and had no accidental administration of this or other drug. Beta-lactams SPT, including piperacillin-tazobactam, were positive to piperacillin-tazobactam (2.5 mg/mL), penicillin G and major and minor determinants. SPT to cefuroxime was negative, but intradermic test was positive (2.5 mg/mL). Available beta-lactams specific IgE determinations were all negative: amoxicillin, ampicillin, penicillin G and penicillin V. The patient has now indication for beta-lactams eviction. After the diagnosis the patient was transferred to the nuclear medicine department to minimize beta-lactams exposure risk and since then no further reactions occurred.

Discussion

Patient's clinical manifestations can be classified as moderate anaphylaxis (9), and as occupational anaphylaxis as the triggers and conditions are only work-environment related (10). This case illustrates how a detailed history is essential in drug allergy workup (11), although drug provocation test (DPT) is the diagnostic "gold standard" due to its finest sensitivity (12). In this patient DPT was not performed, but occupational anaphylaxis diagnosis can be established based in the temporal relationship between piperacillin handling and manifestations, the piperacillin positive SPT (13) and the absence of exposure other than preparation handling. For immediate IgE-mediated hypersensitivity reactions, the presence of drug-specific IgE is usually taken as sufficient diagnostic evidence (11,13). Specific IgE *in vitro* assays are available, although most are not adequately validated (11). We used a validated and indicated *in vivo* methodology (14) and this may be a strength of our study compared to the previously published similar case (8). There is clinically significant cross-reactivity between penicillins, and much less or possibly no clinically significant cross-reactivity between specific penicillins, cephalosporins, and other non-penicillin beta-lactams (15). Piperacillin shares the beta-lactam ring with ampicillin, amoxicillin and cloxacillin (16) and so these antibiotics must be avoided in this patient. The sensitization found to cefuroxime may represent a co-sensitization also due to exposure, or might be associated to beta-lactams cross-reactivity. The clinical relevance of cefuroxime sensitization should be evaluated by DPT. Carbapenems and monobactams are also safely used in individuals with confirmed penicillin allergy (15) and may constitute another alternative.

In this case, sensitization was probably due to occupational nontherapeutic exposure to antibiotics. It can occur by various routes, and contact with spilled drugs and powder or foam inhalation are the most common (4). Cutaneous sensitization is often fast, in weeks or months (2), and was probably enhanced in this patient by a damaged skin barrier leading to local and systemic immune responses (17). Clearly identified risk factors for drug-induced anaphylaxis, like female sex or concurrent medications, do not include professional exposure (1), although some studies point out that HCP seem to have an increased risk of penicillin allergy (18,19). Lifelong avoidance of the drug and cross-reactive drugs is recommended when drug-induced anaphylaxis has occurred (13).

Piperacillin is provided as a powder, and should be dissolved prior to administration. This antibiotic preparation generates more aerosolization than other intravenous antibiotics (8). This patient had anaphylaxis without direct drug contact, suggesting that piperacillin inhalation may be another major route of sensitization or symptoms trigger.

One possible limitation of this case report is the relative uncertainty about sensitization route, although both patient and parents denied recent and frequent therapeutic exposures, as well as in remote past. This question was several times reconfirmed and this is a strength compared to the previously published report where occupational sensitization was assumed only based in occupational exposure (8).

Technical prevention is based on risk elimination, possibly replacing products or substances responsible for allergic manifestations to non-sensitizing agents (2). Allergic risk prevention is essential, and must be rapidly implemented to prevent incapacitating occupational diseases. The authors describe the case report of a health care professional that developed beta-lactams allergy in the context of occupational exposure.

Conflict of interest

The authors declare that they have no conflict of interest.

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Eur Ann Allergy Clin Immunol Vol 50, N 2, 94-96, 2018

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Successful rapid subcutaneous desensitization to anakinra in a case with a severe immediate-type hypersensitivity reaction

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Key words
drug allergy; immediate hypersensitivity; interleukin-1 receptor antagonist; anakinra; immunologic desensitization

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Doi
10.23822/EurAnnACI.1764-1489.30

Summary
Anakinra, one of the novel biological agents, is a recombinant human IL-1 receptor antagonist. It is preferred as an alternative drug for familial Mediterranean fever cases where colchicine is not sufficient or cannot be used due to its side effects. Like all other biologics, hypersensitivity reactions to anakinra are quite rare. This is the first case which was successfully desensitized with anakinra after a severe immediate-type hypersensitivity reaction.

Introduction

Anti-interleukin (IL)-1 agents are one of the biological agents that have increased use in recent years. The anti-IL-1 agent anakinra is a recombinant human IL-1 receptor antagonist that is used as a treatment option in various disorders (1). Despite a good safety profile, local reactions at the injection site are common (2-4). Familial Mediterranean fever (FMF) is an autosomal recessive inflammatory disease in which colchicine is used as the primary treatment (5-7). Anakinra is an alternative drug for FMF cases where colchicine is not sufficient, or colchicine cannot be used due to its side effects (7). Systemic reactions are quite rare with anti-IL-1 agents and they are generally well tolerated (8,9). We present a 38-year-old female who developed an immediate-type hypersensitivity reaction after the use of anakinra and then underwent successful desensitization.

Case presentation

The patient was referred to our clinic by the Department of Rheumatology due to a history of anaphylaxis after the use of anakinra. Her prior medical history revealed that after the diagnosis of FMF a year before, she was prescribed with colchicine at first. However, she had developed nausea, vomiting, cough, dyspnea, wheezing, dysphagia, and fatigue within 30 minutes after ingestion of the first colchicine dose. She had rapid recovery within one hour of treatment, but at the emergency room she was not able to remember the names of the drugs taken. She retried colchicine on her own a week later, and presented to emergency room again with the same reactions. Anakinra treatment was started by the rheumatology department, due to the history of immediate-type hypersensitivity reaction with colchicine and newly diagnosed renal amyloidosis. She again developed vomiting and nausea, dyspnea,

Table I *- Protocol for subcutaneous desensitization with anakinra[1].*

Steps[2]	Dilution	Volume (mL)	Injected dose (mg)	Cumulative dose (mg)
1	1/1000	0.1	0.015	0.015
2		0.3	0.045	0.06
3		0.6	0.09	0.15
4	1/100	0.1	0.15	0.3
5		0.3	0.45	0.75
6		0.6	0.9	1.65
7	1/10	0.3	3	4.65
8		0.6	6	10.65
9	1/1	0.1	10	20.65
10		0.25	25	45.65
11		0.55	55	100.65

[1]Premedication with 5 mg of desloratadine and methyl-prednisolone 40 mg was administered 30 minutes before the desensitization procedure.
[2]Administered at 30-minute intervals.

cough, wheezing, and dysphagia 5 minutes after the first dose of subcutaneous injection of anakinra (Kineret® 0.67 mL [100 mg]; Sobi Inc., Stockholm, Sweden). She was treated with methylprednisolone, pheniramine maleate and oxygen therapy. Her symptoms relieved within one hour.

The patient was referred to our clinic for drug allergy evaluation and desensitization. The results of skin prick and intradermal tests with the undiluted form of Kineret® were all negative. Desensitization with anakinra was planned due to nonexistence of an alternative drug. Although there was no doubt about the typical hypersensitivity reaction, a single blind placebo challenge was performed with physiological saline prior desensitization procedure, to rule out the possibility of a psychosomatic basis. No reaction was observed with the placebo challenge (3 different physiological saline doses of 0.1 cc, 0.5 cc, and 1 cc administered SC with 30 minute intervals). Premedication with desloratadine oral tablet and methylprednisolone 40 mg IV was administered one hour before the desensitization procedure. The initial concentration for the desensitization was 0.1 ml of a 1/1000 dilution of the therapeutic dose (100 mg = 0.67 ml). Cumulative dosing was achieved by SC administrations of increasing doses at 30-minute intervals (**table I**). The desensitization protocol was completed without any reaction. One week later, total injection dose was divided into two and administered SC into separate arms, as local erythema and edema (about 10 cm) developed at the injection site. The patient was able to use anakinra without premedication and without any problems after the desensitization procedure.

Discussion

Interleukin-1 antagonists have been recently used, beyond rheumatoid arthritis, in different disorders, such as FMF (4,6). Anakinra is a safe drug that is generally well tolerated. Local reactions at the injection site are frequently reported, but systemic reactions are quite rare (4,8-10). As far as we know, this is the second case that developed a severe immediate-type hypersensitivity reaction and successfully desensitized with anakinra.

The role of an IgE-mediated mechanism in anaphylaxis is a controversial issue in the instance that a patient has never been exposed to a drug before. On the other hand, some studies show that previous contact with the causative drug is not an obligatory prerequisite for immune-mediated drug hypersensitivity, and cross-reactivity between the involved drug and unrecognized prior exposure to similar chemical structures cannot be ruled out (11). Our patient did not have a prior history of anakinra usage and her skin tests were negative. The timing and type of reaction strongly suggest a non-IgE-mediated immediate-type hypersensitivity mechanism to anakinra.

Desensitization is a safe method of re-administering a drug that causes immediate-type hypersensitivity reactions (12). Desensitization with anakinra has previously been reported in four separate case reports in the literature (10,13-15). Three of these cases were desensitized for a late-onset hypersensitivity reaction, and one case was desensitized for an immediate hypersensitivity reaction with anakinra (13-15). In the first reported case with an immediate-type hypersensitivity reaction (urticaria + angioedema) sensitization to anakinra was shown with skin tests, and the patient was successfully

desensitized (10). However, the reaction was thought to be a mild systemic reaction due to the presence of skin and mucosa findings only. Desensitization was started with 0.1 ml of a 1/100 dilution of anakinra, since starting with higher doses was recommended for cases with mild systemic reactions. Since the reaction intensity of our case was more severe, desensitization was started with more diluted doses and the protocol was completed without any reactions. In conclusion, this is the first case which was successfully desensitized with anakinra after a severe immediate-type hypersensitivity reaction to anakinra. Desensitization can be performed using this protocol in cases with a severe systemic reaction. Total daily dose may be divided into two and administered SC into separate arms, if local reactions develop during follow-up.

Patient consent

Obtained.

Conflict of interest

The authors declare that they have no conflict of interest.

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