Successful Rapid Desensitization to Glatiramer Acetate in a Patient With Multiple Sclerosis

Syrigou E[1], Psarros P[1], Grapsa D[2], Syrigos K[2]
[1]Allergy Department, "Sotiria" General Hospital, Athens, Greece
[2]GPP, Athens School of Medicine, "Sotiria" General Hospital, Athens, Greece

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Glatiramer acetate (GA; Copaxone, Teva Pharmaceuticals) is an injectable peptide mixture with immunomodulatory properties. It is approved by the FDA as a first-line treatment for reducing the frequency of relapse and slowing the progression of disability in the relapsing-remitting form of multiple sclerosis (MS) [1]. A considerable amount of research conducted over the last decade has demonstrated that GA is a clinically effective and well-tolerated drug with a more favorable adverse effect profile than other disease-modifying therapies for MS [1]. However, local skin reactions to subcutaneous injection of GA may occur in approximately 20% to 60% of patients, while up to 10% of patients may develop immediate postinjection systemic reactions, which are mainly characterized by flushing, chest pain, palpitations, dyspnea, throat constriction, and urticaria [2].

To the best of our knowledge, there is only 1 previous study reporting successful desensitization to GA in a small series (6 cases) of patients with MS and GA-associated local or systemic reactions [2]. Herein, we report an additional case of successful rapid desensitization to GA in a patient with MS.

A 51-year-old woman with MS had been treated with daily subcutaneous administration of GA for the previous 4 years without adverse reactions. Four weeks before presenting to us she developed injection site redness and swelling followed by generalized urticaria which was resistant to antihistamines (high dose of levocetirizine 5 mg×4/24h) and corticosteroid therapy (methylprednisolone 60 mg/24h). The patient discontinued GA, as advised by her neurologist, and her urticaria resolved. She was referred to our department for further evaluation.

Skin prick tests (SPTs) and intradermal (ID) skin testing to GA and mannitol (an inactive ingredient of Copaxone with allergenic potential) were performed 4 weeks after the initial hypersensitivity reaction to minimize the risk of false-negative results; a positive reaction was defined as the presence of a wheal with a diameter of at least 3 mm larger than that produced by a negative control (diluent) 20 minutes later. Histamine (10 mg/mL) was used as a positive control. For the SPT, a drop was applied to the volar surface of the forearm. For ID injections, 0.03 mL was injected.

In the case of GA, the patient was tested with concentrations of 1:100 (0.2 mg/mL), 1:10 (2 mg/mL), and 1:1 (20 mg/mL) for the SPTs and of 1:1 000 000 (0.00002 mg/mL), 1:100 000 (0.0002 mg/mL, and1:10 000 (0.002 mg/mL) for the ID tests. Mannitol was tested at a concentration of 100 mg/mL for the SPT and 10 mg/mL for the ID test. SPT showed a borderline reaction to GA (wheal diameter, 3 mm) at a concentration of 20 mg/mL, while ID testing produced a positive reaction at a concentration of 0.002 mg/mL (1:10 000). SPT and ID skin testing with mannitol produced negative results.

After obtaining the patient's informed consent, subcutaneous desensitization to GA was carried out in an outpatient setting under close medical supervision. Increasing doses of GA were administered subcutaneously every 15 minutes, with a starting dose of 0.000002 mg followed by gradual dose escalation up to 11 mg (the exact protocol used is shown in the Table). The entire desensitization procedure lasted 3 hours and 15 minutes and was well tolerated, with no immediate or delayed adverse events. The patient was able to resume daily administration of GA with no recurrence of urticaria or any other hypersensitivity reactions during a follow-up period of 3 months.

The development of postinjection systemic reactions is a well-recognized adverse effect of treatment with GA.

Table. Glatiramer Acetate Desensitization Protocol

Time	Concentration, mg/mL	Volume, mL	Dose, mg	Total Dose, mg
0.00	0.00002	0.1	0.000002	0.000002
0.15	0.0002	0.1	0.00002	0.000022
0.30	0.0002	0.5	0.0001	0.000122
0.45	0.002	0.1	0.0002	0.000322
1.00	0.002	0.5	0.001	0.001322
1.15	0.02	0.1	0.002	0.003322
1.30	0.02	0.5	0.01	0.013322
1.45	0.2	0.1	0.02	0.033322
2.00	0.2	0.5	0.1	0.133322
2.15	2	0.1	0.2	0.333322
2.30	2	0.5	1	1.333322
2.45	20	0.1	2	3.333322
3.00	20	0.35	7	10.333322
3.15	20	0.55	11	21.333322

Associated symptoms are usually mild and self-limiting in nature, but may include a variety of systemic events resembling anaphylaxis; these symptoms seem to be responsible for the majority of withdrawals from treatment [3,4]. Nonfatal anaphylactic reactions, hypothetically triggered by accidental intravenous administration of GA with subsequent histamine release, may also occur on rare occasions [4,5]. Interestingly, the occurrence and severity of these adverse reactions to GA are impossible to predict, do not seem to be associated with a history of an atopic condition, and can develop at any time during continuous treatment, even after a long latency period of several months or even years (as in our reported case) [2,5,6].

Drug-induced hypersensitivity reactions comprise allergic and pseudoallergic reactions (non-immune-mediated hypersensitivities) and some drugs, including GA, may elicit both kinds of responses [2,4,7]. Non-immune-mediated hypersensitivity reactions may imitate the clinical features of IgE-mediated allergic reactions (such as urticaria) without detectable involvement of a specific immune mechanism [7]. The exact effect of GA on the immune system and the factors leading to systemic adverse reactions in a subset of treated patients remain poorly understood [5]. As shown by previous immunologic studies, the majority of patients under GA treatment develop detectable GA-specific IgG4 antibodies but not GA-specific IgE antibodies, most likely as a consequence of the immunomodulatory action of the drug itself (ie, modulation of T_H1 and T_H2 immune responses) [2,8]. It has been suggested that this immunomodulatory action may also explain the rarity of IgE-mediated reactions to GA [2].

Previous data on desensitization procedures for patients with a history of adverse reactions to GA are extremely limited. Bayerl et al [9] reported a failed attempt at desensitization to GA in a patient with previous systemic reactions to the drug. By contrast, Bains et al [2] evaluated the safety and long-term effectiveness of a GA desensitization protocol in 6 patients with MS and GA-associated local or systemic reactions, with a successful outcome in all but 1 case.

The present report provides additional evidence on the safety and effectiveness of desensitization to GA and reinforces the view that this procedure may represent an important therapeutic option in patients with GA-associated hypersensitivity.

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Conflicts of Interest

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Dimitra Grapsa
"Sotiria" General Hospital
Mesogion 152, 115 27
Athens, Greece
E-mail: dimgrap@yahoo.gr

No Cross-Reactivity With Cephalosporins in Patients With Penicillin Allergy

Martinez Tadeo JA, Perez Rodriguez E, Almeida Sanchez Z, Callero Viera A, Garcia Robaina JC

Drug Allergy Unit, Allergy Department, Hospital Universitario Ntra. Sra De Candelaria, Tenerife, Spain

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β-Lactam antibiotics are among the most commonly prescribed antibiotics and are also responsible for the highest proportion of IgE-mediated hypersensitivity reactions [1]. In clinical practice, patients with documented allergy to penicillin are advised to avoid all β-lactam antibiotics, with the treatment limitations that this implies. This paper presents an observational retrospective study evaluating cross-reactivity between penicillin and cephalosporins in our population.

We reviewed all patients studied for suspected β-lactam allergy in the last 2 years in our unit. We selected all patients with penicillin or amoxicillin allergy confirmed by either skin tests or an oral challenge and who underwent an oral challenge with cephalosporins.

We performed skin prick tests (SPTs) and intradermal tests (IDTs) with β-lactam determinants PPL and MDM (Diater SA), penicillin, amoxicillin, and amoxicillin-clavulanic acid. In the event of a negative result, a single-blind placebo-controlled challenge (SBPCC) was performed with the same drug. Patients with a positive skin test or SBPCC underwent skin tests and SBPCC with cefuroxime (SPT, 250 mg/mL; IDT, 10 mg/mL) and/or cefixime (SPT, 200 mg/mL) up to the therapeutic dose. Skin tests (SPT and IDT) were also performed with ceftriaxone (100 mg/mL and 10 mg/mL) and/or ceftazidime (100 mg/mL and 2.5 mg/mL). All cephalosporins were diluted with 0.9% NaCl. For injectable cephalosporins, we used the intravenous form under sterile conditions, and for cefixime we weighed and smashed the tablet and then diluted it with 0.9 % NaCl.

Twenty-two patients (9 men and 13 women) met the inclusion criteria (Table). The median age was 46 years (range, 7-80 years). Ten patients (45%) presented exclusive skin symptoms and 12 (54%) presented associated respiratory or

Table. Skin Test and Oral Challenge Results for Penicillin/Amoxicillin and Cephalosporins

	Age, y	Sex	Drug	Clinical Manifestations	SPT	IDT	SBPCC	Alternatives
1	47	F	A-C	Anaphylaxis	-	Pen, Ax	NP	Cefuroxime, cefixime
2	48	F	A-C	Cutaneous	-	-	+	Cefuroxime, cefixime
3	54	M	Ax	Cutaneous	-	Ax	NP	Cefuroxime, cefixime
4	12	M	A-C	Cutaneous	-	-	+	Cefuroxime, cefixime
5	61	F	A-C	Anaphylaxis	Pen, Ax, A-C	NP	NP	Cefuroxime, cefixime
6	63	M	A-C	Anaphylaxis	Pen, Ax, A-C	NP	NP	Cefuroxime, cefixime
7	35	M	A-C	Anaphylaxis	-	Ax, A-C	NP	Cefuroxime, cefixime
8	36	F	A-C	Anaphylaxis	-	Ax	NP	Cefuroxime, cefixime
9	76	F	A-C	Cutaneous	-	-	+	Cefuroxime, cefixime
10	59	F	A-C	Cutaneous	-	-	+	Cefuroxime, cefixime
11	29	M	A-C	Cutaneous	-	-	+	Cefuroxime, cefixime
12	47	M	A-C	Cutaneous	Pen	Pen, MDM	NP	Cefuroxime
13	33	F	Ax	Anaphylaxis	Ax	NP	NP	Cefuroxime
14	61	F	Ax	Anaphylaxis	Ax	NP	NP	Cefuroxime, cefixime
15	80	F	Ax	Cutaneous	-	-	+	Cefixime
16	43	F	Ax	Anaphylaxis	Ax	NP	NP	Cefixime
17	41	F	Ax	Cutaneous	Ax	NP	NP	Cefixime
18	9	M	Pen	Cutaneous	Pen	NP	NP	Cefuroxime, cefixime
19	47	F	A-C	Anaphylaxis	-	Pen, Ax	NP	Cefuroxime, cefixime
20	61	M	A-C	Anaphylaxis	Ax, A-C	NP	NP	Cefuroxime, cefixime
21	51	F	A-C	Anaphylaxis	Ax, A-C	NP	NP	Cefuroxime, cefixime
22	7	M	Ax	Anaphylaxis	-	Ax	NP	Cefuroxime, cefixime

Abbreviations: Ax, amoxicillin; A-C, amoxicillin-clavulanic acid; F, female; IDT, intradermal test; M, male; NP, not performed; Pen, penicillin; SBPCC, single-blind placebo-controlled challenge; SPT, skin prick test.

cardiovascular symptoms. The culprit drugs were amoxicillin (n=7, 31.81%), amoxicillin-clavulanic acid (n=14, 63%), and penicillin (n=1, 4.54%).

Sixteen patients (72%) were diagnosed by skin tests (9 by SPT and 7 by IDT) and 6 (28%) by SBPCC. Skin tests and SBPCC with oral cephalosporins were negative in all patients. Seventeen patients were challenged with both cefuroxime and cefixime, 2 with just cefuroxime, and 3 with just cefixime. These 5 patients were challenged with just 1 cephalosporin because they could not spend more time in our unit for work reasons. The cephalosporin selected in each case was that prescribed by the patient's family practitioner. Skin tests with ceftriaxone and ceftazidime were also negative in all patients, although a challenge was not performed for logistic reasons

Allergy to penicillins is frequently overestimated by both patients and physicians. Several published series have shown that only 10% of patients studied for adverse penicillin reactions are truly allergic to penicillin [2].

In patients with confirmed allergy to penicillin the question of tolerance to other β-lactams remains unclear. Early in vitro studies suggested high cross-reactivity between penicillin and cephalosporins [3] but these findings have not been reproduced in clinical studies [4].

The antigenic determinants of β-lactam are their β-lactam ring (common to all antibiotics in the group) and side R chains. Specific IgE against these side R chains is thought to be the cause of selective sensitizations to amoxicillin or cephalosporins [5], as the β-lactam ring is related to penicillin allergy.

Certain β-lactam drugs, such as amoxicillin and cefadroxil, share an identical side R chain, resulting in cross-reactivity. Amoxicillin is the most frequently prescribed drug in our setting and the most common cause of sensitization.

In these patients our approach was to test second- and third-generation cephalosporins with a different side R chain to that of amoxicillin [6]. In the case of cefixime, which is available only as an oral drug, we decided to only perform prick tests. Although some authors have performed IDTs with oral preparations with reliable results [7], the ENDA/EAACI Drug Allergy Interest Group Position Paper [8] encourages skin testing with parenteral drugs for better standardization and recommends only SPT for drugs that are available in just tablet, capsule, or topical form. In addition, cefixime has a side chain that is very similar to ceftriaxone (tested in all our patients by SPT and IDT) and positive results with prick tests only have been reported in patients allergic to cefixime [9].

The patient sensitized to both amoxicillin and penicillin tolerated the cephalosporins tested (cefuroxime and/or cefixime), supporting the importance of the side chain and not the β-lactam ring in cross-reactivity between penicillins and cephalosporins [10].

Other third- and fourth-generations cephalosporins (ceftriaxone and ceftazidime) showed negative results in the skin tests, and although good tolerance would be expected, SBPCC should be performed to ensure their safety in penicillin-allergic patients. We believe that our results increase the therapeutic options for patients with penicillin allergy, although further studies with a larger number of patients are needed. In our experience, certain cephalosporins seem to be a safe alternative in patients with penicillin allergy.

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Juan Antonio Martinez Tadeo
Hospital Universitario Ntra. Sra. De Candelaria
Crta. Rosario 145
38010 SC de Tenerife, Spain
E-mail: arkanum78@hotmail.com

Common Variable Immunodeficiency or Late-Onset Combined Immunodeficiency: A New Hypomorphic *JAK3* Patient and Review of the Literature

Abolhassani H[1,2], Cheraghi T[2,3], Rezaei N[2], Aghamohammadi A[2], Hammarström L[1]

[1]*Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden*

[2]*Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*

[3]*Department of Pediatrics, 17th Shahrivar Children's Hospital, Guilan University of Medical Sciences, Rasht, Iran*

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Janus kinase 3 (JAK3)-signal transducer and activators of transcription 5 (STAT5) is a known signaling pathway for the differentiation and survival of hematopoietic lineage cells [1]. This pathway can be activated by binding of ligands to the common gamma chain receptor (γc) and deactivated by Notch signaling, which stimulates ubiquitin-mediated and mitogen-activated protein kinase dependent degradation of JAK3 [2].

The majority of patients with mutations within the catalytically inactive JH2 pseudokinase domain (with mutations in exons 11-17, 45% of reported mutations in the *JAK3* gene) lack expression of the protein, resulting in severe combined immunodeficiency. Typical JAK3-deficient patients are characterized by a phenotype with an absence of T cells (lack of IL-7 signaling) and natural killer (NK) cells (lack of IL-15 signaling). Moreover, B cells are nonfunctional due to the absence of helper T cells or to direct defects in class switch recombination (lack of IL-4 and IL-21 signaling) [3]. Clinically, JAK3 deficiency leads to opportunistic life-threatening infections, intractable diarrhea, failure to thrive in the first months of life, and progressive morbidity and mortality unless stem cell transplantation or gene therapy is performed. However, a selected group of patients present with mild manifestations with various clinical diagnoses, ranging from hypogammaglobulinemia to combined immunodeficiency.

A 14-month-old boy was admitted due to chronic diarrhea. He was the second child of consanguineous parents with a family history of several neonatal deaths on the maternal side due to infections and diarrhea (Figure A,B). He had a medical history of 4 previous hospitalizations because of pneumonia (at 7 months), febrile seizure (at 9 months), viral meningitis (at 11 months), and bronchiolitis and failure to thrive (at 12 months). On physical examination, he was a flappy baby with clenched fists and crepitation over the lower lobes of both lungs. Upper endoscopy showed large

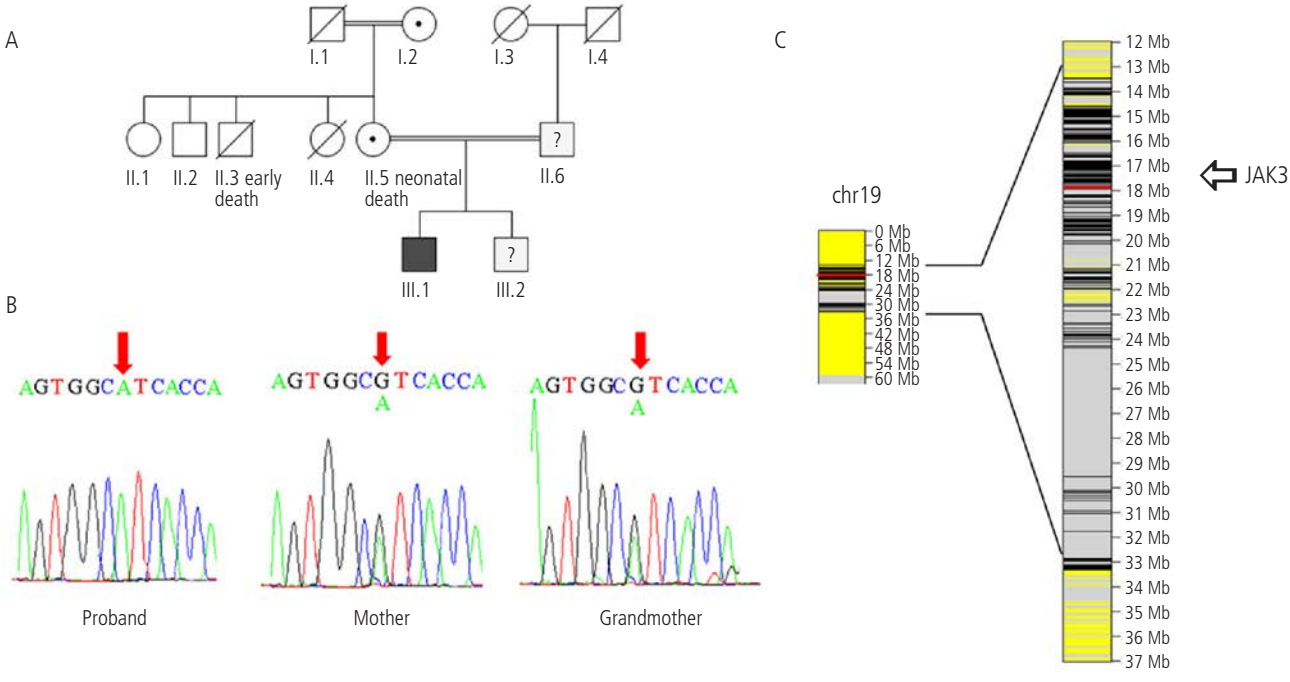

Figure. A, Family pedigree (/, deceased; *double lining*, consanguinity, NA, not applied). B, Sequence analysis of the *JAK3* gene (using the forward primer; the position of the mutation is indicated by a red arrow); homozygous mutation in the proband and heterozygous mutation in the mother and grandmother. C, Homozygosity mapping of a proband using exome sequencng data analysis with AgileVariantMapper sofware (Variant status option: minimum read depth: 5 and hetrozygous cut-off: 10%).

lymphoid aggregations in the duodenum. In the biopsy specimens, surface epithelial cells were shown to contain large focal vascular lymphoid aggregates, mild basal layer hyperplasia, and slight infiltration of eosinophils and plasma cells in the lamina propria. Due to treatment-refractory enteropathy, immunologic evaluation was carried out and a diagnosis of common variable immunodeficiency (CVID) was established (IgG, 100 mg/dL; IgA, 0 mg/dL; IgM, 23 mg/dL; isohemagglutinin titer, 0; white blood cells, 7700 cells/μL, lymphocytes, 2464 cells/μL; $CD3^+$, 1374 cells/μL; $CD3^+CD4^+$, 616 cells/μL; $CD3^+CD8^+$, 665 cells/μL; $CD19^+$, 714 cells/μL; $CD16/56^+$, 167 cells/μL). Intravenous immunoglobulin (IVIG) improved the patient's general condition, and his chronic diarrhea subsided. Subsequent genetic homozygosity mapping suggested linkage of his disease to a 19.2-Mb region on chromosome 19 ($p=3.8\times10^{-28}$, Figure C). As the boy had been born to consanguineous parents, he would be expected to show an autosomal recessive inheritance pattern. Thus, we performed exome sequencing using a previously described method [4] and first filtered out synonymous mutations and eliminated common variant. We then prioritized the results depending on homozygous mutations, primary immunodeficiency–associated genes, and damaging prediction using appropriate software. We found a known, homozygous mutation in p.V722I (c.2164G>A) in the *JAK3* gene. All other known causative gene defects responsible for CVID (including CD19, CD20, CD21, CD81, ICOS, TACI, BAFFR, and LRBA defects) were excluded by the exome sequencing data. Despite regular IVIG administration and prophylactic antibiotics, the patient was admitted at the age of 5 years with respiratory distress, oral candidiasis, bilateral chronic otitis media, and conjunctivitis. His tonsils were atrophic and strabismus was detected. Pulmonary tuberculosis (TB) was suspected on chest X-ray. However, the TB skin test did not produce any indurations and 3 gastric lavages failed to show evidence of TB. A candida skin test was also performed but the result was negative. Complete immunologic tests were subsequently repeated, revealing a mild decrease in the absolute number of NK (75 cells/μL) and T cells (554 cells/μL), with just 0.2% of activated ($CD38^+HLA\text{-}DR^+$) T cells. Antifungal therapy was subsequently added to the therapeutic regimen to cover both candidiasis and probable *Pneumocystis jiroveci* pneumonia. However, the clinical condition of the patient deteriorated and he died due to respiratory failure.

To the best of our knowledge, 3 other patients with a V722I mutation within exon 15 of the JH2 domain (at the

Table. Clinical and Immunological Manifestations of Patients Presenting With a Mild Phenotype of JAK3 Deficiency

Mutation	Allele 1	Allele 2	Domain	Atypical Clinical Presentation	Atypical Immunologic Presentation	References
Het	E481G	K482-S596del	JH2, JH3		Residual function of JAK3, substantial number of NK and $CD4^+$ T cells, normal IgG and IgM, hyper IgE	[3,8]
Hom	Y100C	Y100C	JH7	Age at diagnosis, 5 mo	Normal lymphocytes, T cells, $CD4^+$ cells	[9]
Het	R651W	E694K	JH2		Normal lymphocytes, T cells, $CD4^+$ and $CD8^+$ cells	[7]
Het	P151R	?	JH6	Age at diagnosis, 10 mo	Normal IgM	[9]
Het	M1V	IVS18+3G>C	JH7	Age at diagnosis 72 mo; only diagnosed at this age due to a search for a donor for her brother	Normal IgG, IgA, and IgM	[7]
Het	A58P	C1024fsX1037	JH1, JH7	Age at diagnosis, 19 mo; mild phenotype that did not affect growth	Reduced, but not absent, circulating T cells, normal IgG, IgA, and IgM	[7]
Hom	G589S	G589S	JH2		JAK3 displayed substantial γc binding, high numbers of T and NK cells, CVID presentation	[5]
Het	C193Y	IVS12+3G>T	JH2, JH5	Age at diagnosis, 9 mo; no infectious complications, healthy without bone marrow transplantation and only IVIG therapy	Normal IgG, IgA, and IgM, hyper IgE; only specific antibody deficiency	[2]
Het	C227fsX49	G589D	JH2, JH5		Normal lymphocytes, T cells, $CD8^+$ cells; normal IgG, IgA and IgM; only specific antibody deficiency	[2]
Hom	V722I	V722I	JH2	Age at diagnosis,14 mo; only IVIG therapy	CVID presentation, slightly reduced numbers of T and NK cells present	Current case

Abbreviations: CVID, common variable immunodeficiency; Het, heterozygous; Hom, homozygous; IVIG, intravenous immunoglobulin; IVS, intervening sequence; JH, portion containing domain; NK, natural killer.

loop between aF and aG, leading to a steric clash in the protein core and a misfolded protein) have been reported, but all of them were heterozygous for the mutation and only 1 was shown to be a compound heterozygote (with G987fsX1031), presenting with low numbers of T and NK cells and hypogammaglobulinemia [5]. Our patient is the first case with a homozygous mutation in this position and also the first case of JAK3 deficiency in Iran. The JH2 domain of the JAK3 protein has essential regulatory functions with suppression of kinase activity (JH1 domain) and cytokine-inducible activation of signal transduction (STAT5), resulting in tightly balanced JAK3 activity during normal hematopoietic development. Gain of function mutations in JH2 (of note the V722I activating allele) have also been found in selected hematologic malignancies such as acute megakaryoblastic leukemia and $CD4^+$ mycosis fungoides [6].

Mutations in the JH2 domain may thus be associated with mild immunodeficiency, similarly to some recently identified compound heterozygous mutations in other domains with a weak signaling capacity (Table). This phenotype can often be explained by engraftment of maternal T cells or residual expression and function of the JAK3 protein, or it may reflect a potentially different developmental pathway for $CD4^+$ T lymphocytes [3,8].

Our patient was an atypical case in that he had normal T-cell subsets and NK cells but compromised humoral immunity resembling CVID. This suggests that missense mutations and small in-frame deletions may permit residual kinase activity, receptor binding, or intracellular trafficking with gradually developing dysfunction. Therefore, immunologic studies of T cells and mutation analysis for *JAK3* may be considered for a selected group of CVID patients with an early-onset history of chronic enteropathy, failure to thrive, opportunistic infections, and continued reduction of T-cell subpopulations. Recent next-generation sequencing evaluation of patients previously diagnosed with CVID has shown that different primary immunodeficiency gene defects involving *RAG1* [4], *RAC2, CD27* and *ZBTB24* (unpublished data) can mimic the phenotype of CVID and may thus be misclassified. Other causes of combined immunodeficiency with autosomal recessive inheritance should thus also be considered in patients with early-onset hypogammaglobulinemia mimicking CVID, particularly in a consanguineous family. Thus, a comprehensive, unbiased search for mutations in known primary immunodeficiency genes may be warranted in CVID patients with unusual clinical features.

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Conflicts of Interest

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Lennart Hammarström
Department of Laboratory Medicine
Division of Clinical Immunology and Transfusion Medicine
Karolinska University Hospital Huddinge
SE-141 86 Stockholm, Sweden
E-mail: lennart.hammarstrom@ki.se

Ondansetron Hypersensitivity: A Clinical Diagnosis Protocol and Cross-Reactivity Study

García Núñez I[1], Algaba Mármol MA[2], Reina Ariza E[1]
[1]*Allergy Department, Hospital Quirón Málaga, Malaga, Spain*
[2]*INFOCA Medical Department, Environmental Council, Córdoba, Spain*

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Palabras clave: Ondansetron. Alergia a medicamentos. Test de exposición controlada con medicamentos.

Drug allergies are frequently studied in allergy departments and are the third most common reason for consultation in this setting after rhinitis and asthma [1]. Nonsteroidal anti-inflammatory drugs and β-lactams are the most commonly suspected causes of hypersensitivity reactions in such cases [1], and other drugs are implicated only in a small percentage of patients [2]. Serotonin-receptor antagonist antiemetics are very rarely implicated in drug allergy cases, and very few cases have been described in the literature [3-8].

A 29-year-old woman with nonmetastastic breast ductal carcinoma was evaluated at our allergy department for 2 clinical episodes compatible with allergy symptoms following intravenous premedication with dexamethasone 40 mg and ondansetron 8 mg before chemotherapy. In the first episode, 15 minutes after premedication, the patient experienced vulvovaginal pruritus, which was resolved with dexchlorpheniramine. In the second episode, 10 minutes after premedication, she experienced severe odynophagia with dyspnea and generalized pruritus, which were resolved with several inhalations of salbutamol and budesonide, oxygen therapy, and intravenous dexchlorpheniramine. She was not administered chemotherapy agents in either of the episodes and had never been exposed to serotonin-receptor antagonist drugs before.

After obtaining informed signed consent, we tested methylprednisolone by skin prick testing (SPT) at 20 mg/mL, intradermal testing [IDT] at 20, 2 and 0.2 mg/mL, and a single-blind placebo-controlled oral challenge with the following schedule: placebo-placebo-placebo-2.5 mg-5 mg-12.5 mg-20 mg (cumulative dose, 40 mg), with a 30-minute interval between doses and a 2-hour observation period. The patient was informed that she should go to an emergency department if she developed symptoms in the following 24 hours.

After confirmation of good tolerance to methylprednisolone, we performed SPT with an undiluted concentration and IDT with ondansetron at 2, 0.2, and 0.02 mg/mL; the concentrations were chosen based on previous clinical reports [4,6,7]. The SPT was negative and IDT was positive at 0.02 mg/mL. Ten healthy controls underwent the same tests. All the SPT results were negative, as was the IDT for the 0.02-mg/mL concentration at the immediate (20 minutes) and delayed readings (24 and 48 hours). The results for the higher concentrations (0.2 and 2 mg/mL) were positive in all cases. IDT at 0.02 mg/mL was therefore positive in the patient but not in the control group.

We next performed skin tests with dexamethasone (SPT at 4 mg/mL and IDT at 4, 0.4, and 0.04 mg/mL) and a single-blind placebo-controlled oral challenge with 40 mg dexamethasone according to the following schedule: placebo-placebo-placebo-5 mg-10 mg-25 mg. The patient showed good tolerance in the allergy department and over the next 48 hours.

To study cross-reactivity with other serotonin-receptor antagonists, we performed SPT and IDT with tropisetron, palonosetron, and granisetron (undiluted for SPT and 0.1, 1, and 10 mg/mL for IDT). The results were negative for granisetron in all cases and positive for palonosetron and tropisetron in the IDTs at 1 mg/mL and 10 mg/mL. The same tests were performed in the same control group as above and they were negative in all cases except for the IDT with a concentration of 10 mg/mL (positive in all controls). Granisetron tolerance was confirmed by performing a single-blind placebo-controlled oral challenge with the following schedule: placebo-placebo-placebo-0.5 mg-1 mg-1.5 mg [6,7].

Ondansetron hypersensitivity is very uncommon in our allergy department and hypersensitivity studies are based on clinical results only, without confirmation of diagnosis by highly sensitive or specific in vitro studies. In the case described, we based the skin tests performed on previous reports [4,6,7], with the inclusion of a healthy control group, because the optimal concentrations of this drug have not been established. We also tested irritant concentrations in the patient and in the control group in order to confirm previously reported findings [4,6].

Skin tests were performed to evaluate cross-reactivity between serotonin-receptor antagonists in order to identify a possible alternative treatment for our patient. Similar tests have been performed by other groups for granisetron [3,6,7], but not for palonosetron or tropisetron. Because of the positive results obtained with palonosetron and tropisetron, these drugs were not tested in an oral challenge. Although there is a report of a patient sensitized to ondansetron who tolerated palonosetron [9], our case is very different. Cross-reactivity between ondansetron and serotonin-receptor antagonists tested was a possibility because our patient had never taken these drugs before and the healthy controls had negative skin test results with the same concentrations. The negative skin test results for granisetron gave us the opportunity to test granisetron with a high percentage of good results in safety and tolerance. Our results indicated cross-reactivity between ondansetron, palonosetron, and tropisetron, and, in accordance with previous reports [3,6,7], we confirmed good tolerance to granisetron.

In our patient, the association between corticosteroids and antiemetic drugs and a clinical report compatible with drug hypersensitivity suggested an initial diagnosis of corticosteroid hypersensitivity, but this was ruled out following skin tests and an oral challenge with the implicated corticosteroid and a corticosteroid from another pharmacological group [10].

To conclude, before offering an alternative drug to a patient with a drug allergy, it is necessary to first study possible cross-reactivity using skin tests and challenge tests. It is also important to avail of a detailed clinical report to contemplate all possible diagnostic hypotheses and to confirm or discard these after an in vivo and/or in vitro study. In our patient, we demonstrated good tolerance to granisetron (alternative

to odansetron if necessary) and cross-reactivity between ondansetron, palonosetron, and tropisetron, and discarded corticosteroid allergy.

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Conflicts of Interest

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Ignacio García Núñez
Departmento de Alergía
Hospital Quiron Malaga
Avda Imperio Argentina nº 1
29004 Malaga, Spain
E-mail: h62ganui@hotmail.com

Epigenetic Regulation Analyses of *FOXP3* in Olive Pollen Allergy

Calzada D[1]*, Aguerri M[1]*, Baos S[1], Lahoz C[1,2], Cárdaba B[1,2]
[1]*Immunology Department, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain*
[2]*CIBERES, CIBER de Enfermedades Respiratorias, Spain*
**Calzada D and Aguerri M have contributed equally to this article.*

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Palabras clave: Epigenética. FOXP3. Inmuno-regulación. Metilación. Alergia al polen de olivo.

Regulatory T cells (Tregs) are essential for the induction and maintenance of immune tolerance to allergens [1]. Forkhead winged-helix transcriptional factor (FOXP3) has been identified as a specific molecular marker for Tregs, and its expression is considered essential for the formation and function of these cells. Its absence, by contrast, is associated with severe allergic inflammation and high IgE levels [2]. Many molecular mechanisms have been described that could modulate FOXP3 expression [3], and several groups have observed that epigenetic regulation is crucial for controlling the expression of the *FOXP3* locus [4-5].

In 2007, an evolutionary conserved CpG-rich element within the *FOXP3* locus was identified as being selectively demethylated in natural Treg (nTreg) cells, but not in FOXP3-CD4+CD25- or conventional T cells. This Treg-specific demethylated region (TSDR) was also associated with modified histones in nTregs, but not in FOXP3– T cells, strengthening the idea that epigenetic mechanisms contribute to the control of *FOXP3* gene expression. Demethylation of TSDR has been associated with the stability of *FOXP3* expression [6]. Several studies have demonstrated that the methylation status of *FOXP3* is implicated in several diseases such as systemic sclerosis [7]. In the case of allergies, this mechanism appears to contribute to clinical immune tolerance during peanut oral immunotherapy [8].

Olive pollen allergy is one of the major causes of allergic diseases in Mediterranean countries and some regions of Australia and North America. Olive pollen induces mainly nasal and conjunctival symptoms, although it may induce asthma exacerbations in areas with high airborne levels of pollen.

We recently demonstrated that peripheral blood mononuclear cells from olive pollen–allergic patients showed a decrease in a regulatory cytokine, TGF-β, and that this decrease correlated with low levels of FOXP3 expression compared with nonallergic controls [9]. These findings led us to study the DNA methylation of *FOXP3* as a possible mechanism responsible for the differences found in FOXP3 expression between untreated olive pollen–allergic individuals and healthy controls, specifically, in a region called TSDR, which had been proposed as an important methylation-sensitive element regulating FOXP3 expression [10].

The population studied was composed of 39 individuals (22 nonallergic individuals and 17 untreated olive pollen–allergic patients) selected from a previous immunological study [9] and recruited at the allergy departments of hospitals in Andalusia, a region in Spain with particularly high pollen counts during the pollen season (5000 grains/m^3, with peaks reaching more than 10 000 grains/m^3).

Olive pollen–allergic patients fulfilled the following inclusion criteria: seasonal rhinitis and/or asthma from April to June, a positive skin prick test to *Olea europaea* pollen extract (ALK Abelló), and no previous immunotherapy.

The exclusion criteria were current smoking and less than 10 years´ residence in the study area (Andalusia). Informed consent was obtained from all study participants and ethical approval for the study was obtained from the ethics and research committees of all the participating hospitals. Biological samples were obtained from Biobanco-FJD, IIS-Fundación Jiménez Díaz Madrid.

Mean (SD) total IgE and *O europaea*–specific IgE antibodies were significantly higher (P<.05) in allergic patients (166.76 [160.67] and 21.47 [19.61], respectively) than in controls (71.03 [91.60] and 0.34 [0.00], respectively).

Mean *FOXP3* expression levels determined by quantitative real-time polymerase chain reaction (qRT-PCR) were significantly decreased in untreated olive pollen–allergic individuals as compared with the control group, supporting previous findings [9].

For the DNA methylation study of TSDR (320 bp, X-chromosome 49117047-49117367, positive strand) (genome.ucsc.edu), we designed a methylation-specific PCR assay to study 14 CpG islands. Amplification primers were based on previously described primers [5] but with some modifications introduced using Pyromark Assay Design Software 2.0 (Qiagen). A schematic view of the study design is shown in Figure A. Specifically, genomic DNA was isolated from whole blood samples extracted from untreated allergic patients (n=22) and controls (n=17) using BioRobot EZ1 DSP Workstation (Qiagen); the samples were extracted during the olive pollen season. Bisulfite conversion of this genomic DNA was performed using MethylCode Bisulfite Conversion (Invitrogen). PCR was performed in a final volume of 50 µL containing 1xPCR Buffer (Promega), 1.25 U Go Taq DNA polymerase (Promega), 200 µM dNTPs, 1.5 µM MgCl2, 12.5 pmol of each primer (forward and reverse), and 15 ng bisulfite-treated genomic DNA. DNA without bisulfite conversion and PCR reactives without DNA were used as negative controls. PCR consisted of an initial denaturation step of 95ºC for 5 minutes, followed by 40 cycles of 95ºC for 1 minute, 55ºC for 1 minute, and 72ºC for 1 minute, and a final step of 72ºC for 10 minutes. PCR products were analyzed on

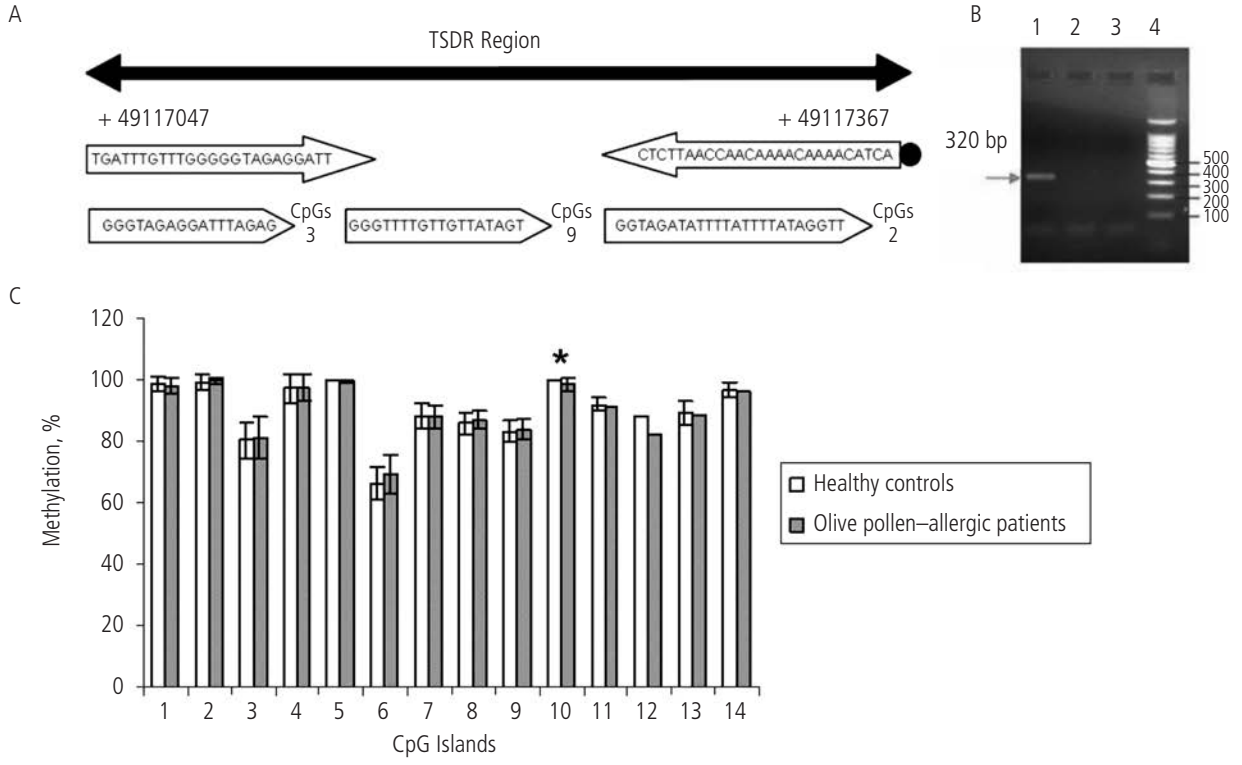

Figure. A, Schematic overview of the TSDR region and the amplicon designed for methylation analysis. Upper arrows show forward and biotinylated reverse primers. Lower arrows represent sequencing primers. B, Agarose gel analysis of polymerase chain reaction (PCR) products: 1. PCR products obtained from bisulfite-treated genomic DNA. Negative controls: 2. Non-bisulfite-treated genomic DNA, 3. PCR control (without DNA). 4. 100 bp DNA ladder (Promega). C, Methylation profiles of olive pollen–allergic patients and healthy controls. The panel shows the mean percentage of methylation for each CpG island in the 2 study groups. The asterisk shows statistically significant differences. TSDR indicates regulatory T-cell–specific demethylated region; PCR, polymerase chain reaction.

2.5% agarose gels stained with ethidium bromide. The primers designed resulted in correct specific PCR amplification of the bisulfite-treated DNA of the TSDR region (320 bp) (Figure B). After amplification, CpG methylation status was analyzed by pyrosequencing technology using 3 sequencing primers (Figure A) designed by Pyromark Assay Design, Software 2.0. The methylation index for each sample was calculated as the mean percentage for all CpG islands examined. Figure C shows the methylation status profile of the 14 CpG islands studied. No clearly distinctive *FOXP3* methylation profile was observed in either of the 2 study groups. Both groups had heavily methylated islands in the TSDR region, but the methylation status of 1 CpG (number 10) was significantly increased in controls compared with allergic patients. This result was unexpected because we had suspected that the downexpression of FOXP3 in allergic patients would be associated with a greater hypermethylation status than in healthy controls. Hypomethylation status of the TSDR region is usually associated with stable FOXP3 expression, although several authors have reported that demethylation of the TSDR corresponds to FOXP3 expression stability (as in nTreg cells), whereas T cells that express FOXP3 only transiently (TGF-β-induced Treg cells and recently activated conventional human T cells) have a methylated TSDR [4]. One of the major reasons for the lack of differences found could be that the DNA analyzed was extracted from peripheral blood samples and not from purified DNA from Tregs or T cells. This experimental design was based on the approach that we previously used to correlate methylation degree with *FOXP3* gene expression [9], but the data reflect that it is not an optimal strategy for studying *FOXP3* methylation.

In conclusion, our data show that analysis of the degree of methylation in the TSDR-*FOXP3* region using DNA extracted from peripheral blood is unable to explain the decrease in FOXP3 described in olive pollen–allergic individuals, although it does not exclude the role of methylation in FOXP3 stability.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Blanca Cárdaba
Departamento de Inmunología
IIS-Fundación Jiménez Díaz
Avda Reyes Católicos Nº 2
28040 Madrid, Spain
E-mail address: bcardaba@fjd.es

Septic Arthritis and Atopic Dermatitis: 2 Cases and a Review of the Recent Literature

Kusunoki T[1], Shimozono F[2], Maruki M[2], Futami T[2], Fujii T[1]
[1]*Department of Pediatrics, Shiga Medical Center for Children, Shiga, Japan*
[2]*Department of Orthopedics, Shiga Medical Center for Children, Shiga, Japan*

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Palabras clave: Dermatitis atópica. Caso clínico. Artritis séptica. *Staphylococcus aureus*. Revisión.

In atopic dermatitis (AD), skin barrier disruption and reduced antiseptic protection are known to enable skin infection, which can become invasive [1]. However, no reports to date have specifically focused on joint infection associated with AD. We report 2 cases of septic arthritis of the hip joint associated with severe AD and review recently published cases.

Case 1

A 3-month-old boy with a 2-month history of eczema was brought to our clinic after his parents noticed that he had not moved his right leg since the previous day. The patient's body temperature was 37.5ºC and he had severe maculopapular, erythematous rash on the face, trunk, and outer aspects of all 4 extremities. The leukocyte count was 22 800/mm^3, C-reactive protein (CRP) level was 3.4 mg/dL, IgE was 22.2 U/mL, specific IgE to egg white was 3.50 kU$_A$/L, and thymus- and activation-regulated chemokine was 3306 pg/mL. He was diagnosed with AD based on the typical eczematous lesions and laboratory findings. Magnetic resonance imaging showed edema in the right hip and surrounding soft tissue, and a blood culture was positive for methicillin-sensitive *Staphylococcus aureus*. The patient was diagnosed with septic arthritis of the right hip. His condition resolved completely after joint irrigation and intravenous infusion of cephalosporin antibiotics.

Case 2

An 11-year-old boy with eczema since infancy had been diagnosed with AD. He had no other allergic diseases. His eczema had worsened, and he could not sleep well because of itching. He was referred to our clinic with a 5-day history of progressively increasing pain in the left hip. His body temperature was 38ºC. He had dry skin all over his body and lichenification on the flexor aspect of both elbows and knee joints, as well as on the inguinal and lumbar regions. The leukocyte count was 7700/mm^3, CRP level was 11.3 mg/dL, IgE was 1300 U/mL, and thymus- and activation-regulated chemokine was 717 pg/mL. Specific IgE was positive to house dust mite and several pollen allergens. The magnetic resonance findings in the left hip were the same as those found in case 1. MRSA was found in fluid from the hip joint, venous blood, and skin lesions. Antibiotic sensitivity was the same as for the strains found in the hip and the skin. This patient also recovered completely after joint irrigation and intravenous infusion of cephalosporins.

In both cases, treatment with hydration, a moisturizing ointment, and a topical corticosteroid ointment markedly improved the skin symptoms. This improvement has since been maintained thanks to regular management at the clinic.

Our experience with these 2 patients prompted us to review recently published cases in which AD was associated with septic arthritis or osteomyelitis. PubMed and the Japan Medical Abstract Society website were searched for case reports of septic arthritis/osteomyelitis associated with AD since 2000. We found 23 cases in the literature (2 in the English-language literature [2,3] and the remainder in the Japanese-language literature). The characteristics of the 25 cases, including the 2 cases reported here, are summarized in the Table. Age was widely distributed (median, 5 years), and most patients were male (male-to-female ratio of 1.5). The most frequently affected site was the hip joint (14 cases), followed by the knee joint (4 cases), sacroiliac joint (3 cases), and femur (3 cases). *S aureus* was the causative pathogen in 18 cases, with 5 cases involving methicillin-resistant *S aureus*. According to the descriptions in the literature (eg, generalized dermatitis with desquamation and numerous scratch marks [2] and eczematous skin lesions with some lichenoid portions on the hand [3]) and based on the definition for severity of AD in the Japanese guidelines [4], the severity of the skin lesions was considered moderate to severe in 19 cases. Prognosis was relatively good, with complete recovery in 22 cases, while sequelae were observed in 3 cases. All the cases reviewed here, including the 2 reports from the English-language literature, involved Japanese patients.

Nakamura et al [5] observed AD in 4 of 16 children (25%) with acute septic arthritis. Hashi et al [6] found 1 case of AD in 5 patients (20%) with septic arthritis. Likewise, during the last 5 years, we have encountered 9 cases with septic arthritis and AD, including the 2 cases in the present study (22.2%). AD was moderate to severe in all cases. The frequency of moderate to severe AD in patients with septic arthritis was higher than the frequency of AD among Japanese children shown in the guidelines (only 2% for moderate to severe cases) [4], suggesting a strong relationship between AD and septic arthritis.

The surface of atopic skin is massively colonized by *S aureus*. Scratching itchy skin enables bacteria to enter the bloodstream, resulting in invasive infection at metaphyses [1]. Subsequent invasion of the adjacent joint may result in arthritis. Although the *S aureus* strains found in the joint and the skin were not identified, the same antibody sensitivity pattern in case 2 suggests that they are identical. The hip joint is generally the most affected site, although knee involvement is more common in children [7]. A high degree of suspicion is necessary in hip joint arthritis, because there is often no obvious joint swelling and signs and symptoms are nonspecific, especially in infants and young children [7]. The reason for the predominance of Japanese patients is unclear. However,

Table. Clinical Characteristics of 25 Cases of Septic Arthritis/Osteomyelitis Associated With Atopic Dermatitis

Age, y	Sex	Site	Pathogen	Suspected Severity of AD	Prognosis	Reference
13	Male	Right sacroiliac	MSSA	Moderate-severe	Good	Ohno et al, 2000[a]
15	Female	Right hip	SA	Moderate-severe	Good	Kitamura et al, 2000 [2]
3	Male	Left hip	Unknown	Unknown	Good	Ueda et al, 2001
Infant	unknown	Knee	SA	Moderate-severe	Unknown	Ono et al, 2003[a]
5	Male	Right knee	MRSA	Moderate-severe	Unknown	Hidaka et al, 2004[a]
12	Male	Right hip and knee	SA, MRSA	Moderate-severe	Good	Yamagata et al, 2004
3 mo	Female	Left hip	MSSA	Moderate-severe	Good	Kimura et al, 2005
5	Male	Left hip	MSSA	Moderate-severe	Good	Nakamura and Fujioka, 2006 [5]
21	Female	Left sacroiliac	MSSA	Unknown	Good	Moriwaki et al, 2006
10 mo	Male	Right knee and femur	MRSA	Moderate-severe	Contracture	Nakamura and Fujioka, 2006 [5]
6 mo	Female	Right hip	MSSA	Moderate-severe	Good	Nakamura and Fujioka, 2006 [5]
7 mo	Male	Right hip	beta Str	Moderate-severe	Growth disturbance	Nakamura and Fujioka, 2006 [5]
11	Male	Left hip	Unknown	Unknown	Good	Hiyane et al, 2007[a]
1	Female	Left knee	MSSA	Mild	Good	Matsushita et al, 2008
3	Male	Left tibia	Unknown	Mild	Growth disturbance	Nagai et al, 2008
3	Male	Left femur	MSSA	Moderate-severe	Good	Nagai et al, 2008
11 mo	Female	Left hip	MSSA	Moderate-severe	Good	Nagai et al, 2008
7	Female	Right knee	SA	Moderate-severe	Good	Suzuki et al , 2009[a]
23	Female	Right hip	Group B Str	Unknown	Good	Kinugasa et al, 2009[a]
31	Male	Cervical vertebrae	SA	Moderate-severe	Good	Tsutsumi et al, 2010
5	Female	Right hip	MRSA	Moderate-severe	Good	Hashi et al, 2012 [6]
12	Male	Right hip	MRSA	Moderate-severe	Good	Yamagata et al, 2012[a]
15	Male	Right sacroiliac	Group A Str	Moderate-severe	Good	Yasuda and Nisimatsu, 2012 [3]
3 mo	Male	Right hip	MSSA	Moderate-severe	Good	Case 1, 2013
11	Male	Right hip	MSSA	Moderate-severe	Good	Case 2, 2013

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; SA, *Staphylococcus aureus;* Str, *Streptococcus*.
[a]Meeting abstract.

ethnic differences in susceptibility are not likely, considering non-Japanese case reports published before 2000 [8,9].

Treatment of eczema with appropriate use of corticosteroids and/or tacrolimus ointment not only improves symptoms, but also reduces the number of colonizing bacteria [10]. As septic arthritis is a potential complication of AD, patients should receive appropriate topical therapy.

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Conflicts of Interest

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Takashi Kusunoki
Department of Pediatrics, Shiga Medical Center for Children
5-7-30 Moriyama, Moriyama, Shiga 524-0022, Japan
E-mail: kusutaka@gamma.ocn.ne.jp

Kiwifruit Anaphylaxis: The Usefulness of Molecular-Based Allergy Diagnostics

Tosca MA[1], Pistorio A[1], Accogli A[1], Silvestri M[1], Rossi GA[1], Ciprandi G[2]
[1]Instituto Giannina Gaslini, Genoa, Italy
[2]IRCCS - Azienda Ospedaliera Universitaria San Martino, Genoa, Italy

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Palabras clave: Kiwi. Anafilaxia. Síndrome de alergia oral. Diagnóstico molecular.

Kiwifruit allergy (KA) is a classic example of a new food allergy resulting from modified dietary habits. Progressively increasing consumption of kiwifruit started in 1970, and the first allergic reaction was described in 1981 [1]. Since then, many cases and series have been reported. KA can be an isolated manifestation (monoallergy) or occur in association with pollen or latex allergy. Symptoms vary considerably, and severity ranges from mild and localized oral allergy syndrome (OAS) to life-threatening anaphylaxis [2]. Lucas et al [3] performed a thorough study of the clinical features of KA in a large group of patients: symptoms affecting the oral mucosa were the most frequent (65%), although 18% of patients experienced more severe symptoms (respiratory and cardiovascular). Of note, symptoms were more severe in young children than in adults, and children were more likely to react to the first known ingestion.

The diagnostic workup for KA is typically based on a clinical history, detection of allergen-specific IgE by skin prick test and/or in serum, and oral challenge. Prick-by-prick testing with fresh kiwifruit is significantly more sensitive (83%-100%) than testing with commercial skin test extracts [4], although it has low specificity (31%) and lacks standardization. Oral food challenge is the mainstay for diagnosis of food allergy, as it accurately demonstrates the causality of the specific allergen, and double-blind, placebo-controlled, food challenge (DBPCFC) is the gold standard. However, challenge testing is very dangerous in patients with anaphylaxis. Thus, determination of serum allergen-specific IgE could be used to confirm KA allergy. The crude allergen extract has been available for some time. Molecular-based allergy diagnostic techniques have recently become available in clinical practice [5] and make it possible to define and characterize the sensitization profile, thus revealing potentially dangerous proteins and providing a more accurate prognosis [5]. Eleven kiwifruit allergens have been identified to date. The first was the cysteine protease actinidin (Act d 1), which is the major kiwifruit allergen and is frequently associated with monosensitization [6]. Act d 2 is a member of the thaumatin-like protein family [7,8]. Kiwellin (Act d 5) is a cell wall protein involved in ripening. Act d 8 is a Bet v 1 homolog, and Act d 9 is a kiwifruit profilin: both allergens are specific

for pollen-kiwifruit–allergic patients. Act d 10 is a nonspecific lipid transfer protein, and Act d 11 is a member of the major latex protein family.

Bublin et al [9] evaluated a very specific population of patients with KA and positive DBPCFC results to kiwifruit and found that the use of a panel of individual kiwifruit allergens, including Act d 1, 2, 3, 4, 5, 8, and 9, had a relevant impact on the sensitivity of in vitro diagnosis of KA [9]. In addition, the use of component-resolved diagnosis (ie, molecular-based techniques) enabled patients to be classified according to their sensitization profile: Act d 1 is a potential marker for isolated KA, whereas Act d 8 and Act d 9 could indicate typical cross-reactivity patterns [9].

The results of a recent study showed that kiwifruit allergen sensitization patterns differ across Europe. In particular, Le et al [10] found that sensitization to Act d 1 and living in Iceland were strong risk factors for severe KA. Given that this study was conducted for scientific purposes in a selected population of patients using a large allergen panel, we thought that it would be interesting to evaluate the sensitization profile in children with KA in a real-life setting. In this regard, we retrospectively studied a group of children seen during the last year at Istituto Giannina Gaslini, a tertiary-level children's hospital in northern Italy. We subdivided the patients into 2 groups: children with a history of anaphylaxis after ingestion of kiwifruit and children with OAS to kiwifruit. Diagnosis of kiwifruit anaphylaxis was based on the demonstration that ingestion could induce anaphylaxis. The diagnosis of anaphylaxis was based on 3 validated criteria. First, acute onset of an illness involving skin and/or mucosal tissue and at least 1 of the following: respiratory compromise; reduced blood pressure or associated symptoms of end-organ dysfunction. Second, 2 or more of the following rapidly after kiwi ingestion: involvement of skin and/or mucosal tissue; respiratory compromise, reduced blood pressure, and persistent gastrointestinal symptoms. And third, reduced blood pressure after ingestion in individuals with known KA.

OAS to kiwi was diagnosed based on validated criteria, such as suggestive history (immediate occurrence of local symptoms after ingestion), sensitization confirmed by serum specific IgE and food challenge test results. Specific IgE to raw kiwifruit allergen was measured using the ImmunoCAP platform (Thermo Fisher Scientific), with positivity defined as an IgE level >0.35 kU_A/L. Specific IgE to the molecular components of kiwifruit (eg, Act d 1, Act d 2, Act d 5, and Act d 8) was determined using the semiquantitative ISAC method (Thermo Fisher Scientific): IgE >0.30 ISU was considered a positive result. Oral challenge testing was performed openly, according to the procedures stated in the PRACTALL consensus [11].

We studied 46 patients: 14 with anaphylaxis and 32 with OAS. The Table shows the clinical and immunologic characteristics of the 2 groups. Age was significantly lower in the anaphylaxis group. Total and allergen-specific IgE levels to raw kiwi did not differ significantly between the 2 groups. Children with anaphylaxis had higher IgE levels to Act d 1 than children with OAS. On the other hand, the median values of IgE to Act d 2, Act d 5, and Act d 8 were under the positivity cutoff in both groups.

Receiver operating characteristic analysis showed that the Act d 1 ISAC cutoff level of >0.8 ISU was fairly reliable (area under the curve 0.77; 95%CI, 0.58-0.9) with good sensitivity (76.9%) and specificity (78.9%). The diagnostic odds ratio was 12.5 (95%CI, 2.3-68.2), thus highlighting the relevance of this finding.

Our findings are partially consistent with those of the European survey on KA by Le et al [10], although they are not consistent with those of other studies, in which the detectable level of IgE to the molecular components of kiwifruit is lower, possibly because of the limitation imposed by the semiquantitative nature of ISAC.

Nevertheless, our findings could be clinically relevant, as they confirmed that the sensitization profile was dependent on the geographic area and that the ISAC cutoff (>0.8 ISU) made it possible to confirm a severe reaction to kiwifruit.

Therefore, we believe that molecular-based diagnosis of allergy could be useful in the workup of children with KA, mainly when a cutoff point can be defined to identify severe reactions.

Table. Demographic and Immunological Parameters in Children With Anaphylaxis or Oral Allergy Syndrome to Kiwi[a]

	Anaphylaxis	Oral Allergy Syndrome	*P*[b]
Age, y	5.4 (3.4-7.8)	7.6 (6.1-10.5)	.008
Total IgE, kU/L	897 (222-2010)	544.5 (168.5-1529)	.50
Raw kiwi IgE, kU_A/L	2.9 (0.7-8.4)	0.8 (0.2-2.6)	.12
Act d 1, ISU	2 (1-3.4)	0.1 (0.1-0.8)	.008

[a]All values are shown as median (IQR).
[b]Mann-Whitney test.

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Conflicts of Interest

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Giorgio Ciprandi
Largo R. Benzi 10
16132 Genoa, Italy
E-mail gio.cip@libero.it

A Case of Heparin Allergy With Good Tolerability to Fondaparinux During Pregnancy

Buonomo A[1,*], Nucera E[1,*], De Carolis S[2], De Stefano V[3], Pascolini L[1], Di Pasquo E[2], Betti S[3], Schiavino D[1]
[1]*Department of Allergy, Università Cattolica del Sacro Cuore, Rome, Italy*
[2]*Department of Obstetrics and Gynecology, Università Cattolica del Sacro Cuore, Rome, Italy*
[3]*Department of Haematology, Università Cattolica del Sacro Cuore, Rome, Italy*
[*]*Both authors contributed equally to the manuscript*

Key words: Unfractionated heparin allergy. Low-molecular-weight heparin allergy. Fondaparinux cross-reactivity. Pregnancy.

Palabras clave: Alergia a heparina no fraccionada. Alergia a heparina de bajo peso molecular. Reacción cruzada de fondaparinux. Embarazo.

Fondaparinux sodium is a synthetic pentasaccharide that binds strongly to antithrombin and enhances inactivation of factor Xa without interacting with factor II or platelets. Fondaparinux is as efficacious as low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH) in the prevention of venous thromboembolism and in the treatment of deep vein thrombosis (DVT) and pulmonary embolism (PE) [1].

Although heparins are widely used, hypersensitivity reactions are not frequent. The most common reactions involve cell-mediated hypersensitivity and are characterized by itchy erythematous plaques at the injection site and, occasionally, by maculopapular exanthema. Heparin-induced thrombocytopenia (IgG-mediated type II reaction) and IgE-mediated reactions are rare. Cross-reactivity between LMWHs is high and can also involve UFH [2]. Fondaparinux is the treatment of choice for DVT and PE and for the prevention of venous thromboembolism when LMWH and UFH are contraindicated, as is the case in patients who develop hypersensitivity reactions, since it has very low cross-reactivity with both LMWH and UFH [3-5].

Fondaparinux has been shown to be safe in several published case reports and case series, even during pregnancy [6-9], although the package insert for fondaparinux still reports "it should not be prescribed during pregnancy if not necessary."

We report the case of a 40-year-old woman with essential thrombocythemia and a clinical history of intrauterine fetal death and recurrent PE.

The patient was diagnosed with essential thrombocythemia when she was 20 years old and started antiplatelet therapy with ticlopidine. At age 29, her first pregnancy (during which she was taking aspirin) ended in intrauterine fetal death at the 28th week. One year later, she had a PE with no evident cause and started cytoreductive treatment with interferon combined with aspirin. At 30 years of age, she had a miscarriage. During this pregnancy, she was receiving LWMH (nadroparin calcium) and aspirin. Three months later, she had another PE.

Soon after diagnosis of the second PE, the patient restarted treatment with nadroparin calcium (Seleparina) and a week later developed local urticaria, generalized itching, cough, and dysphagia a few minutes after intake. One month later, she was again treated with nadroparin calcium (Fraxiparina) and developed the same symptoms after the first dose. In both cases, symptoms resolved after treatment with oral antihistamines and betamethasone.

The patient was then referred to our allergy unit and underwent skin prick and intradermal testing with sodium heparin, nadroparin calcium, bemiparin sodium, reviparin sodium, dalteparin sodium, and fondaparinux sodium [2].

Immediate-type skin testing produced a wheal and flare reaction for all the drugs except fondaparinux. Skin tests were all negative at the 48-hour reading. These results were consistent with IgE-mediated allergy to both UFH and LMWH. At the end of the procedures, the patient underwent a challenge test and tolerated 2.5 mg of subcutaneous fondaparinux with no side effects.

At age 34 years, the patient became pregnant. Warfarin was stopped immediately and she started treatment with fondaparinux 2.5 mg once daily and aspirin 100 mg once daily.

The second trimester ultrasound scan revealed no major malformations, although 3 areas of amnion-chorionic detachment were found (the largest was 141×10 mm). Therefore, low-dose aspirin was stopped. Ultrasound controls were performed, and at 32 weeks of gestation, only a single area of amnion-chorionic detachment (56×31 mm) was detected; no major bleeding or hypersensitivity reactions were observed.

A cesarean section was performed at 36 weeks of gestation. Fondaparinux was stopped 24 hours before surgery, and the intraoperative blood loss was 400 mL. The patient delivered a healthy girl weighing 2280 g (18th percentile on a national standard curve). The newborn had no complications (platelet count of 460×10^9/L) and was discharged on the sixth day after delivery. The patient was discharged from the maternity ward 4 days after the birth. Daily administration of 2.5 mg of fondaparinux was restarted after 12 hours after surgery and continued for another 40 days. No major bleeding was observed during the puerperium.

Heparins are used during pregnancy to prevent complications such as fetal loss, intrauterine growth restriction, and early-onset pre-eclampsia and to prevent thromboembolic events in high-risk patients.

Cell-mediated hypersensitivity reactions to heparins usually manifest as itchy erythematous plaques at the injection site; maculopapular exanthemas can be also observed if treatment is not stopped promptly. IgE-mediated hypersensitivity reactions are less frequent. Reported symptoms include pruritus, urticaria, rhinitis, bronchial asthma, and anaphylaxis. In these cases, both UFH and LMWH should be avoided and replaced with lepirudin, argatroban, and danaparoid [10].

Fondaparinux sodium is a synthetic pentasaccharide that has low cross-reactivity with UFH and LMWH and is usually well tolerated in patients with hypersensitivity reactions [10].

Even if it is not recommended during pregnancy, fondaparinux has proven successful as prophylaxis in pregnant women with hypersensitivity reactions to UFH and/or LMWH, although no allergy testing results are available [6-9].

In the present case, we performed skin tests and highlighted an IgE-mediated reaction to nadroparin calcium with cross-reactivity to other heparins. Skin tests with fondaparinux were negative, thus confirming the lack of cross-reactivity with other heparins. However, even if the negative predictive value of skin tests with drugs is high, a subcutaneous challenge test under medical supervision is mandatory to confirm tolerability.

Despite these favorable reports on tolerability of fondaparinux in pregnancy, we should bear in mind that the drug can pass the placental barrier in vivo, resulting in measurable antifactor Xa in umbilical cord blood. Even if the concentration in umbilical cord blood is below the concentration required for effective anticoagulation, a potential hazardous effect cannot be ruled out [10].

In conclusion, fondaparinux is effective in pregnant patients with heparin hypersensitivity, although larger studies are needed to confirm safety and tolerability. We recommend allergy testing in patients with type I or type IV hypersensitivity reactions to rule out cross-reactivity before treatment with fondaparinux and thus avoid unexpected reactions.

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Alessandro Buonomo
Allergy Department
Università Cattolica del Sacro Cuore
Largo Gemelli, 8
00168 Rome
Italy
E-mail: alessandrobuonomo@hotmail.com

Vespa velutina nigritorax: A New Causative Agent in Anaphylaxis

Chugo S[1], Lizaso MT[1], Alvarez MJ[1], Arroabaren E[1], Lizarza S[2], Tabar AI[1,3]
[1]*Servicio de Alergología, Complejo Hospitalario de Navarra, Pamplona, Spain*
[2]*Servicio de Alergología , Hospital Donostia, San Sebastian, Spain*
[3]*IdiSNA, Instituto de Investigación Sanitaria de Navarra, Spain*

Key words: Hymenoptera venom allergy. Anaphylaxis. *Vespa velutina.*

Palabras clave: Alergia a veneno de himenópteros. Anafilaxia. *Vespa velutina.*

Allergic reactions to hymenoptera venom range in severity from local reactions to anaphylaxis. Hymenoptera venom is the most frequent cause of anaphylaxis, and up to 31% of cases under hospital care are caused by hymenoptera sting [1]. The incidence of fatal reactions varies from 0.03% to 0.48% per million inhabitants per year. However, mortality after hymenoptera sting might be higher, since some unexplained sudden deaths could be attributed to anaphylaxis.

Apis mellifera, Vespula vulgaris, and *Polistes dominulus* are the most frequent sources of hymenoptera venom in our environment. Sting by these insects is particularly problematic in apiarists owing to poorer tolerance to immunotherapy, a high incidence of new stings in this collective, and a higher prevalence of allergic reactions after new stings [2].

We present the case of 71-year-old amateur beekeeper with no relevant medical history who was tolerant to bee stings. After being stung on the head by a wasp that the patient identified as "velutina", he immediately experienced paresthesia, genital pruritus that rapidly became generalized, hives on the chest, feeling of impending doom, and fainting. He was taken to a health center, where scattered hives and hypotension were observed. A peripheral line was inserted to administer antihistamines and corticosteroids while awaiting the arrival of the ambulance. According to the medical report, no adrenaline was administered.

The patient subsequently underwent an allergy workup after he was shown several specimens of insects. He identified *V velutina nigritorax*. He had previously been stung twice by *V velutina*, although the reaction only occurred at the third sting.

The allergy workup comprised the following tests:

- Skin test (intradermal test) with *A mellifera* (positive, 1 µg/mL), *V vulgaris* (positive, 0.1 µg/mL), and *P dominulus* (positive, 1 µg/mL).
- Total IgE: 3.47 kU/L and serum tryptase: 7.64 µg/L, both using ImmunoCAP (Thermo Fisher Scientific).
- IgE reactivity to complete extract and components (ImmunoCAP, Thermofisher Scientific): *A mellifera*, 1.87 kU_A/L; *Vespula* species, 0.18 kU_A/L; *P dominulus*, 0.12 kU_A/L; *Vespa crabro*, 0.19 kU_A/L; *Dolichovespula maculata*, 0.28 kU_A/L; *Dochivespula arenaria*,

0.19 kU_A/L; rApi m 1, 0.28 kU_A/L; rVes v 1 <0.01, kU_A/L; rVes v 5, 0.82 kU_A/L; and rPol d 5, 0.44 kU_A/L.
- Specific IgE against antigen components using the Advia Centaur platform (Siemens): rApi m 1, <0.01 kU_A/L; rVes v 1, <0.01 kU_A/L; rVes v 5, 0.80 kU_A/L; rPol d 5, 0.40 kU_A/L.
- Specific IgE against antigen components using ImmunoCAP ISAAC 112 (Thermo Fisher Scientific) in the 112-allergen version (*ISAC112*) which includes rApi m 1, nApi m 4, rVes v 5, and rPol d 5. The result was negative (<0.3 ISU-E for the 4 determinations), even though the physician responsible for the study reported that microarray imaging showed slight fluorescence to Ves v 5 that did not reach the positive range.

The wasp *V velutina* (Vespidae family) is from Asia. It reached other continents as a stowaway on ships and spread naturally. The species arrived in the United States of America from Asia in 1840 and can now be found throughout the USA and Canada. It was first found in Europe at the end of 2005 in southern France [3], even though it probably arrived in Bordeaux, France in a wood container 1 year previously. Since then, *V velutina* has spread naturally throughout 32 departments in France, reaching Guipúzcoa, Spain in 2011. In Navarre, Spain it has attacked hives, causing damage and social concern [4]. Experts believe this wasp will have colonized the whole Iberian Peninsula in 10 years.

V velutina is a large insect, and the queen can reach 40 mm in length; the remaining wasps reach 30 mm. They are dark in color and have a velvety, blackish-brown thorax and brown abdominal segments with thin yellow stripes and brown legs with yellow tips. They feed on proteins, which are obtained from caterpillars, butterflies, flies, dragonflies, spiders, and other insects, including bees.

The wasps usually live in forest environments, but they also colonize urban areas. Geographic dispersion is mainly along rivers, and growth is between spring and autumn. They make their nests on treetops in forested areas. The nests are round and up to >40 cm in diameter. The outer covering is made of the pulp of 5-6 leaves separated by 5-10–mm air chambers with a single opening that is usually 1.5 cm in diameter and protected by a small paper roof.

The life cycle begins in spring. The males come out in summer, when the copulation period starts, and leave the nest in autumn.

We report our first case of grade IV systemic reaction after a *V velutina* sting in an apiarist who was previously tolerant to bee venom. We used conventional techniques such as skin tests and specific IgE determination with complete extracts, as well as detection techniques such as Advia Centaur and ImmunoCAP. We verified positivity for vespid allergen antigen 5 and differences between techniques, as reported elsewhere [5].

Molecular diagnosis can help to avoid diagnostic errors due to panallergens, enables identification of genuine sensitization to a specific source of allergy, and, as in the present case, reveals the existence of cross-reactivity [6].

The detection of specific IgE for antigen 5 from other vespids (Ves v 5 and Pol d 5) suggests the presence of antigen 5 in *V velutina* that cross-reacts with the counterparts of the most common vespids. The question that remains to be answered is whether available immunotherapy for these counterparts could be effective in this patient with anaphylaxis after *V velutina* sting (as reported elsewhere [7]), since treatment with velutina venom is not marketed.

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Conflicts of Interest

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Ana Isabel Tabar
Complejo Hospitalario de Navarra
CS Conde Oliveto
31003 Pamplona (Navarra)
Spain
E-mail: ana.tabar.purroy@navarra.es

Allergy Workup for Suspected Folic Acid Hypersensitivity

Schrijvers R[1,2], Chiriac AM[1], Demoly P[1]
[1]*Exploration des Allergies, Département de Pneumologie et Addictologie, Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, France; and Sorbonne Universités, UPMC Paris 06, UMR-S 1136, IPLESP, Equipe EPAR, Paris, France*
[2]*Internal Medicine, Department of Microbiology and Immunology, University Hospitals Leuven, KU Leuven, Leuven, Belgium; Laboratory of Clinical immunology, KU Leuven, Leuven, Belgium*

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Hypersensitivity reactions to an essential food component such as folic acid are rare. We report the case of a 53-year-old woman who presented with anaphylactic shock after intake of a folic acid supplement. We also provide an overview of the literature and a work plan for suspected folic acid allergy.

Folic acid is composed of a nonreduced aromatic pteridine ring linked to para-aminobenzoic acid and 1 or more glutamic residues. Mean dietary intake of folic acid in adults is around 247-291 µg per day and consists mainly of the polyglutamate conjugate. Synthetic folic acid contains only the monoglutamate conjugate, resulting in higher bioavailability and bypassing the need for removal of the polyglutamate conjugate at the brush border. In the enterocyte, monoglutamate folic acid is reduced, methylated, and released into the bloodstream as 5-methyltetrahydrofolic monoglutamate. However, these mechanisms are saturable, and unmetabolized synthetic folic acid can be detected in blood at doses as low as 200 µg. In blood, metabolization occurs after cellular uptake in much the same way as after intravenous administration of folic acid.

A 53-year-old woman presented with an anaphylactic shock (facial angioedema, nausea, diarrhea, dyspnea with desaturation, and hypotension of 65/35 mmHg [compared with 110/80 mmHg under resting conditions]) 10 minutes after oral intake of folic acid (5 mg, commercially available formulation), prednisone (1 mg), vitamin B_1 (250 mg) and B_6 (35 mg) complex, and a complex of activated charcoal (140 mg), magnesium oxide (180 mg), and simethicone (45 mg). She was treated with antihistamines, corticosteroids, volume expanders, and an adrenaline injection. She reported that the combination was tolerated the previous day but was not completely certain. However, she reported milder symptoms (pruritus, flush, diarrhea, and need to lie down to recover) after consuming beverages and food fortified with vitamins (including folic acid). She had a long history of chronic urticaria treated with antihistamines on demand (thus complicating the interpretation of skin test results), and asthma during childhood. She was not atopic. Baseline serum tryptase was normal (3.9 µg/L; reference value, <13.5 µg/L),

Table. Case Reports of Folic Acid Hypersensitivity[a]

Reference	Age, y, Sex	Clinical History	Product	Skin Test Results	Provocation Test Results
Mitchell et al, 1949 [9]	35, F	Maculopapular dermatitis after 2 weeks of treatment, resolving upon discontinuation Presumed anaphylaxis (flush, dyspnea, tachycardia, anxiety), resolved spontaneously	Folic acid po Folic acid 50 mg IV	IDT+ folic acid	NP
Chanarin et al, 1957 [1]	35, F	Anaphylaxis (itch, rash, malaise, dyspnea, thoracic pain)	Folic acid 5 mg po Folic acid IV	IDT+ folic acid (0.5 mg/mL up to 1:100) IDT– folinic acid (0.25 mg/mL)	NP
Mathur, 1966 [10]	0.75, U	Generalized urticaria (2 episodes)	Folic acid 5 mg po	IDT+ folic acid	NP
Woodliff et al, 1966 [9]	36, F	Generalized urticaria, pruritus (2 episodes, 1 treated with adrenaline and antihistamines)	Folic acid po	IDT+ folic acid (0.1 mg/mL) IDT+ folinic acid (0.1 mg/mL) IDT– methotrexate (0.1 mg/mL)	NP
Sesin et al, 1979 [8]	36, M	*1*) Pruritus *2*) Febrile episode, pruritus, chills, generalized pain, urticaria	*1*) Folic acid 1 mg po *2*) Folic acid 1 mg po (rechallenge 3 months later)	IDT+ folic acid (10 mg/mL)	NP

Dykewicz et al, 2000 [2]	32, F	Anaphylaxis (urticaria, facial angioedema, nausea and vomiting 20 minutes after intake)	Multivitamin tablet	SPT+ folic acid (5 mg/mL, up to 1:10 000) SPT+ folinic acid (5 mg/mL, up to 1:1,000) SPT+ THF (5 mg/mL, up to 1:1,000) SPT+ M-THF (5 mg/mL, up to 1:100) SPT– methotrexate (5 mg/mL) SPT– for folic acid and congeners in 5 controls.	OPT+ for folinic acid (10 - 50 - 100 mg), generalized urticaria
Sanders et al, 2004 [3]	61, F	Recurrent angioedema, urticaria, anaphylaxis	Multivitamin tablet, fortified green vegetables, orange juice, fortified cereals	SPT+ folic acid (0.05 mg/mL) SPT– for vitamin B_1, B_2, B_3, B_6, A, C, D, E, K SPT– folic acid in 1 control	OPT+ with food containing 400 µg of folic acid, urticaria
Smith et al, 2007 [4]	U, F	*1*) Rash, diarrhea, itchy throat (treated with antihistamines) *2*) Anaphylaxis (itchy throat, pruritus, nausea treated with antihistamines and adrenaline) *3*) Anaphylaxis (rash, vomiting, light headedness treated with antihistamines and adrenaline)	*1*) Folic acid 5 mg po *2*) Water with 16 mg folic acid *3*) Feijoa drink with 53.5 µg/mL folic acid	IDT+ folic acid (0.05 mg/mL) IDT– in 1 control	OPT+ (double blind) at 160 µg, urticaria
Pfab et al, 2007 [7]	44, F	Tachycardia, rash, and dyspnea immediately after intake (4 similar episodes)	Multivitamin tablet, fortified juice and sweets (common elements were vitamin B_6, B_{12}, and folic acid)	SPT+ multivitamin tablets SPT– other multivitamin agents, vitamin B_6 and B_{12}	OPT– for excipients (titanium dioxide, magnesium stearate, E127, E131, E132, E151, E172, gelatin) OPT+ multivitamin tablets (1/4 of a Vaso-loges pill, 1/10 of a Dreisafol pill, resulting in generalized urticaria and anaphylaxis, respectively)
Valdivieso et al, 2009 [5]	72, F	Chronic urticaria and occasional facial angioedema (over 8 months)	Folic acid 400 µg po per day	SPT– folic acid (1 and 5 mg/mL)	OPT+ folic acid (0.25 mg - 0.5 mg -1 mg, single blind), generalized urticaria and angioedema. OPT– placebo, excipients. OPT+ folinic acid (50 mg IM), pruriginous macula after 1 h (no reaction at injection site)
Roy et al, 2012 [6]	29, F	Generalized pruritic rash occurring 3 days after intake (multiple episodes)	Multivitamin tablet B_{12}, (B_6 and folic acid 5 mg)	NP	NP
This report	52, F	Anaphylactic shock, milder symptoms after vitamin fortified beverages and food	Folic acid 5 mg and fortified beverages and food	SPT+ folic acid (1 mg/mL up to 1:1000) SPT– folic acid in 8 controls SPT– folinic acid (1 mg/mL) SPT– methotrexate (5 mg/mL)	OPT– glutamic acid

Abbreviations: IDT, intradermal test; M-THF, 5-methyl-tetrahydrofolate; IM, intramuscular; IV, intravenous; NP, not performed; OPT, oral provocation test; SPT, skin prick test; THF, tetrahydrofolate; U, unreported.

[a]We were unable to retrieve data from Sparling et al [6].

thus practically ruling out underlying mastocytosis. The results of skin prick tests (SPTs) to several dilutions of the crushed folic acid pill were positive (wheals of 7 mm and 4 mm at 1 mg/mL and a 1:1000 dilution, respectively), while remaining negative in 7 controls. To exclude the role of excipients, we performed SPTs with an intravenous multivitamin preparation containing folic acid that did not contain any of the excipients present in the pill, thus demonstrating positivity in the patient (wheal 5 mm, folic acid 0.4 mg/mL) but negativity in 4 of the controls. SPTs and oral provocation tests (OPTs) for prednisone and the vitamin B_1-B_6 complex were negative. Activated charcoal, magnesium oxide, and simethicone were not tested owing to the absence of absorption. The results of SPTs for folinic acid (1 mg/mL), methotrexate (5 mg/mL), and a vitamin drink containing folic acid (0.3 µg/mL) were negative. Next, sensitization to 1 of the components of folic acid was evaluated. The results of SPTs for different parabens and monoglutamate sodium were negative. An OPT for the latter was also negative, although natural food sources contain both monoglutamate and polyglutamate folic acid forms. The patient received an allergy card and rescue medication in case of accidental ingestion and was advised to avoid foods and beverages fortified with folic acid.

Since synthetic folic acid became available in 1945, there have been 13 reports of presumed folic acid hypersensitivity including the present one (Table). Reactions have been reported after both oral administration [1-8] and intravenous administration [1], with some occurring after as little as 400 µg of folic acid [5], although even lower reactive doses can be anticipated in the case of fortified foods, as in the present case. The clinical picture ranges from recurrent angioedema, urticaria, and presumed chronic urticaria [3,5] to anaphylaxis presenting immediately after exposure [1-4,7]. A febrile episode with pruritus, urticaria, and generalized pain has also been reported [8], as has a case of episodic delayed pruritic rash occurring 3 days after the intake of a multivitamin preparation [6]. Interestingly, all cases but 1 were female.

The diagnostic workup consists of SPT and intradermal tests (IDTs), which may or may not be followed by an OPT. SPTs were positive in 3 out of 4 cases [2,3,5,7], and a negative SPT result followed by a positive OPT result was recorded in a patient with a suggestive clinical history [5]. IDTs were positive in 5 out of 5 cases [1,4,8,9]. Three patients with a positive SPT result for folic acid also had a positive OPT result [3,4,7]. Together with those of the present case, these results indicate that SPT and IDT using a serial dilution of folic acid with inclusion of controls is mandatory and can be followed by an OPT in the case of a negative skin test result. The role of in vitro tests in the diagnosis of folic acid hypersensitivity remains experimental. Dykewicz et al [2] demonstrated the presence of specific IgE for human albumin bound to folic acid but not folic acid or human albumin alone, suggesting that folic acid might act as a hapten in an IgE-mediated response. However, Valdivieso et al [5] could not detect the presence of specific IgE to folic acid in a patient with a positive OPT result, even though the SPT result was negative and in vitro testing methods differed from those of Dykewicz et al.

Clinical and immunological cross-reactivity with folic acid congeners such as folinic acid or methotrexate should be evaluated to provide a potential alternative for substitution therapy in the case of folinic acid and to rule out cross-reactivity for the others. However, the findings in the different case reports are heterogeneous. SPT positivity for folic acid but not folinic acid [1] and positivity for SPTs [2] or OPTs [5] for both folic acid and folinic acid have been reported. Cross-reactivity with methotrexate was never demonstrated [2,9]. In the present case, SPTs did not reveal cross-reactivity. However, given the severity of the reaction, the intermittent intake of antihistamines potentially skewing results in the case we report, and the cross-reactivity reported in the literature, an additional OPT would be indicated when a congener such as folinic acid is necessary.

The mechanism of folic acid hypersensitivity remains elusive. In the present case, we hypothesize an IgE-mediated reaction to synthetic folic acid entering the bloodstream in an unmetabolized form owing to gastrointestinal enzyme saturation upon overexposure. In the case of necessary supplementation with folic acid, a search for an alternative source such as folinic acid should be undertaken, given the cross-reactivity reported [2,5]. The data we report emphasize the need for a high index of suspicion and additional testing when vitamin supplements or fortified foods are potentially involved in an episode of anaphylaxis.

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Conflicts of Interest

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Rik Schrijvers
Herestraat 49
B-3000 Leuven, Belgium
E-mail: rik.schrijvers@uzleuven.be