## Life-threatening Reaction to Iron Dextran: Protocol for Induction of Tolerance

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Adverse reactions to iron dextran are usually delayed and consist of joint pain, vomiting, nausea, headache, and fever. Life-threatening reactions to iron dextran have been reported [1,2].

We report the case of a 48-year-old woman who experienced a severe reaction to iron dextran and who was able to receive the drug after undergoing a series of tolerance induction protocols.

The patient had celiac disease and could not absorb oral iron; therefore, she required parenteral iron. At the time of her first reaction, she had been pretreated with 10 mg of prednisolone and 10 mg of loratadine 1 hour before administration of parenteral iron. After receiving 300 mg of iron dextran she experienced a severe reaction consisting of maculopapular rash and hypotension. Administration was stopped. As part of the allergy workup, we performed a skin prick test and intradermal test with ferrous sulfate, ferrous lactate, and iron sucrose based on a previously reported protocol [3]. The results were negative.

Because the patient needed iron and there was no alternative route of administration, she was treated with iron dextran utilizing the protocol of Altman et al [4], with some modifications. The patient received the following drugs and doses: intravenous methylprednisolone 50 mg at 13 hours, 7 hours, and 1 hour before administration of iron dextran; intramuscular diphenhydramine (50 mg) and ephedrine (25 mg) 1 hour before iron dextran; and 20 mL of intravenous dextran (150 mg/mL) immediately before iron dextran. The modification consisted of 180 mg of fexofenadine without ephedrine. The target dose was approximately 2 g in 3 days. At the beginning of the protocol, iron dextran (50 mg in 100 mL of normal saline [NS]) was administered intravenously at 20 mL/h. Subsequent doses were administered at 22 hours (100 mg in 250 mL of NS at 40 mL/h), 28 hours (200 mg in 250 mL of NS at 50 mL/h), 47 hours (400 mg in 250 mL of NS at 60 mL/h), 52 hours (500 mg in 250 mL of NS 60 mL/h), 71 hours (500 mg in 250 mL of NS at 80 mL/h), and 75 hours (250 mg in 250 mL of NS at 80 mL/h).

The patient tolerated the first and second day with the increasing doses of iron dextran. On the third day, after the third hour of the infusion (60 mg/h), she experienced a maculopapular vasculitic reaction and edema in her legs. We stopped the protocol and treated the reaction with antihistamines and systemic corticosteroids. The skin lesion had resolved completely 24 hours later (Figure).

We then adjusted the protocol several times until we were able to administer iron dextran in NS every 3-4 months without a reaction. Our protocol required 10 doses, administered as follows: first dose, 0.1 mg in 0.5 mL; second dose, 0.2 mg in 1 mL; third dose, 0.5 mg in 2.5 mL; fourth dose, 1 mg in 5 mL; fifth dose, 2 mg in 10 mL; sixth dose, 5 mg in 25 mL; seventh dose, 10 mg in 50 mL; eighth dose, 20 mg in 100 mL; ninth dose, 50 mg in 250 mL; tenth dose, 100 mg in 500 mg. The total dose was 188 mg in 944 mL of NS. The first 7 doses were administered continuously for 15 minutes each. The eighth dose was administered over 30 minutes, the ninth over 60 minutes, and the tenth over 90 minutes (total time 4 hours and 45 minutes).

Administration of iron is sometimes necessary, even when the patient experiences severe reactions. Altman et al [4], reported the case of a 26-year-old man with chronic malabsorption and gastrointestinal blood loss resulting in iron deficiency anemia and anaphylaxis to iron dextran. Hickman et al [5] reported the case of a 58-year-old woman who developed irritable bowel syndrome after subtotal gastrectomy and severe iron deficiency anemia because of intolerance to impaired absorption of oral iron. The patient experienced a life-threatening reaction after intravenous infusion of iron. The same protocol was used in both cases, and the patients were able to receive 2 g of iron dextran without experiencing a reaction.

In the case we report, serum ferritin levels were 6 ng/mL (15-186 interval range) and hemoglobin 7 mg/dL before the protocol of Altman et al [4] was applied. During administration, the patient received a total dose of 800 mg of iron dextran. After



Figure. A, Skin reaction during the protocol. B, Patient's skin 24 hours after infusion of iron dextran.

2 months, ferritin levels were 106.39 ng/mL and hemoglobin levels were 14 mg/dL, although we were unable to reach our goal of 2 g because the patient experienced a severe reaction. We had to modify the protocol several times before we were finally able to administer iron dextran without a reaction. The patient has subsequently received iron dextran on 3 occasions without experiencing new reactions.

It is difficult to define the mechanism or mechanisms involved in the reaction observed in the present case. Skin test results were negative, although the low sensitivity of the test prevents us from ruling out the involvement of IgE. Other potential mechanisms include formation of antibodies against dextran and a T-cell response or mast cell activation. Slow administration after premedication reduces the severity of a reaction by any of these mechanisms, although it is sometimes necessary to adjust the dose according to the patient's tolerance.

*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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## Perception of Bronchodilation Assessed by Visual Analog Scale in Asthmatics: A Real-life Study

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The visual analog scale (VAS) is a psychometric response scale used to assess subjective characteristics or attitudes in numerous diseases. The continuous (or "analog") aspect of the VAS differentiates it from discrete scales such as the Likert scale. The validity of the VAS was previously documented in the measurement of asthma symptoms in adults and children in both experimental and clinical studies [1-4].

Reversibility of airflow obstruction is pathognomonic of asthma, although bronchodilation (BD) testing is usually performed only in specialized clinics. We hypothesized that the patient's perception of his/her breathing assessed using the VAS was correlated with the response to BD. It was recently reported that the patient's perception of breathing assessed using the VAS might be considered an initial tool for assessment of the response to BD in children with asthma, mainly in those with overt bronchial obstruction [5]. Thus, in the present study, we investigated whether assessment of the patient's perception of breathing using the VAS could be useful when evaluating the response to BD testing in adults with asthma.

We performed a cross-sectional study of 73 consecutive patients with asthma (28 males; mean age, 39.1 years). The local ethics committee approved the study procedure, and all patients gave their written informed consent.

The inclusion criterion was a documented diagnosis of asthma, according to the GINA guidelines (htpp://www.ginasthma.org). The exclusion criteria were recent asthma exacerbation and the presence of acute upper and/or lower respiratory infections. Patients discontinued use of long-acting and short-acting bronchodilators, respectively, 12 and 4 hours before BD.

Spirometry was performed using a computer-assisted spirometer (Pulmolab 435-Spiro 235, Morgan; predictive values based on ECCS 1993) according to guidelines [6,7]. BD testing was performed with 400 μg of salbutamol; airflow obstruction was considered reversible if an increase in $FEV_1$ of at least 12% and 200 mL over baseline was achieved [7].

The VAS evaluation system comprised a 10-cm segment, upon which the patient is asked to indicate his/her perception of breathing by marking a point along the segment [7]. In this study, 0 corresponded to the worst breathing, while 10 corresponded to optimal, symptom-free breathing. The segment was without interval markers.

The Wilcoxon signed rank test was applied to assess any difference between the pretest and post-test VAS score. A receiving operator characteristic (ROC) curve was used to define a predictive optimal cutoff for the percentage increment in the VAS score for detecting positive BD response. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp).

Airflow obstruction was reversed after BD testing in 24 patients. The patients were therefore subdivided into those with a positive response (BD+) and those with a negative response (BD–).

The VAS score both at baseline and after BD testing was significantly higher in BD– patients than in BD+ patients. The difference in the VAS score was lower in the BD– group (1.1 [1.5]) than in the BD+ group (2.1 [1.9]) (Figure). In both groups, a significant increase in the VAS score was observed after BD ($P<.001$).

The ROC curve analysis revealed a predictive optimal increase in the VAS score of 12.5% for BD+ patients. Sensitivity was 83.33%, specificity 55.10%, diagnostic accuracy 64.38%, and the area under the curve 0.71 (0.58-0.83) ($P=.004$) (Figure, D). The positive likelihood ratio was 1.86 and the negative likelihood ratio was 0.30, with a statistically significant ($P=.003$) diagnostic odds ratio of 6.14 (1.83-20.62). Therefore, patients with an increase in the VAS score of ≥12.5% had a 6-fold greater probability of a positive response than those with an increase of <12.5%.

Most studies that have investigated perception of respiratory symptoms were conducted in experimental settings, for example, patients with symptoms induced by bronchoconstrictor stimuli. Very few studies have evaluated the response to BD testing using the VAS score [4,5,8]. The first study to investigate the perception of airway obstruction induced by methacholine challenge and BD testing was
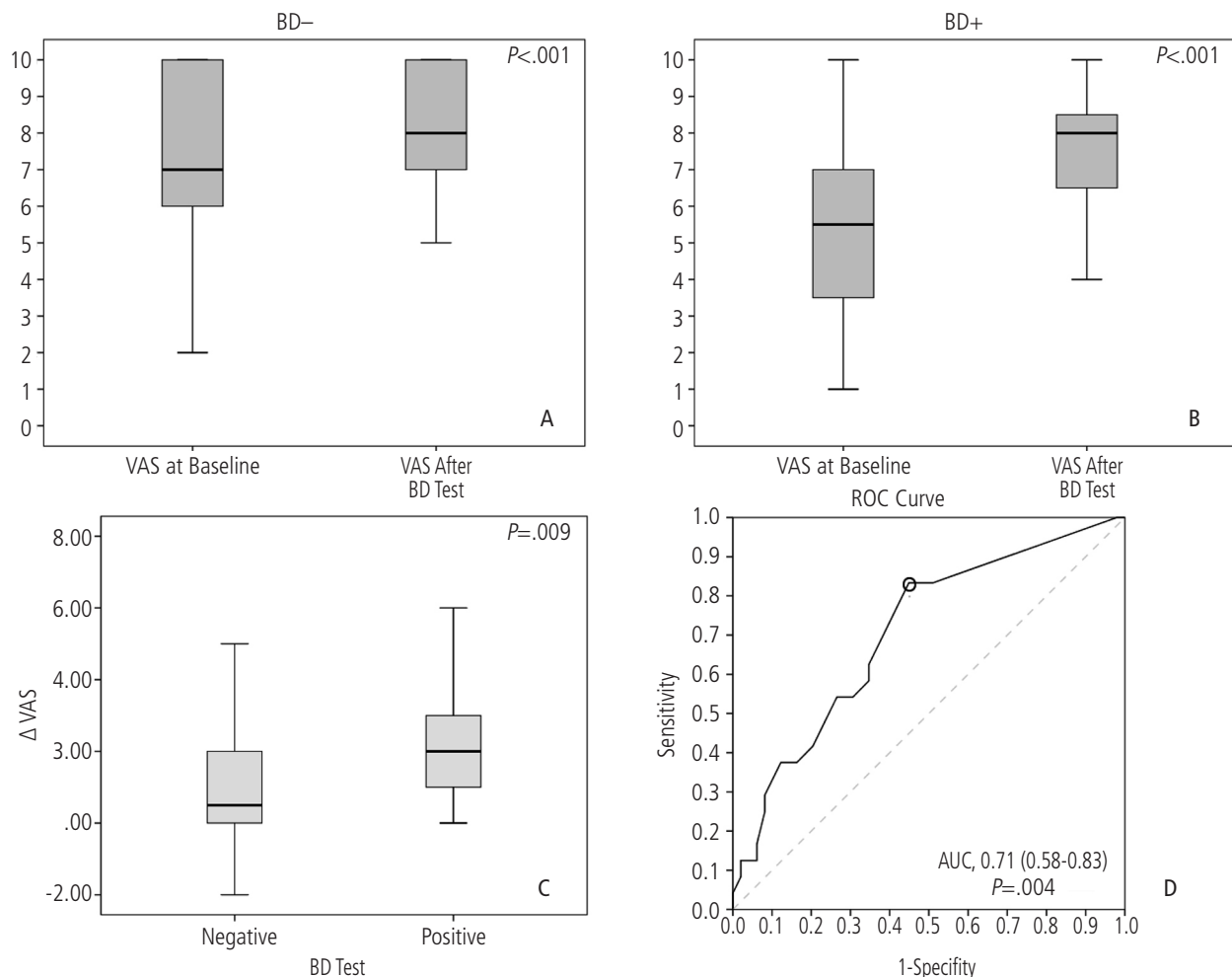


Figure. A, VAS values at baseline and after BD testing in patients with a negative response (BD–). B, VAS values at baseline and after BD testing in patients with a positive response (BD+). C, Difference in VAS values after BD testing in patients with a negative or a positive response. D, ROC curve for defining a cut-off VAS value to predict the response to BD testing. VAS indicates visual analog score; BD, bronchodilation; ROC, receiver operating characteristic.

performed by Baker et al [8]. The second study was performed on adult patients with allergic rhinitis [4]. A more recent study conducted in asthmatic children revealed that assessment of the patient's perception of breathing using VAS was a reliable tool for predicting bronchial reversibility [5]. Therefore, the present study was designed to confirm the possibility of using VAS to assess the response to BD testing in adult asthma patients in a real-life setting, namely, consecutive patients referred to an asthma clinic.

Our findings showed that VAS values increased significantly after BD in all patients. BD+ patients had more impaired lung function than BD– patients. This functional difference was well perceived by the patients: in effect, BD+ patients reported lower baseline VAS values than BD– patients. Interestingly, a significant increase in the VAS values was perceived after BD, even though there was a significant difference in the VAS score between the groups. This finding may prove to be clinically relevant, as it underlines the subjective ability to perceive variations in breathing.

The most important outcome of the present study was the definition of a cut-off value for changes in the VAS score after BD that enables us to predict bronchial reversibility. In this regard, an increase of 12.5% may reliably predict reversibility of airflow obstruction (area under the curve, 0.71; odds ratio, 6.14).

Assessment of BD testing using the VAS could make it possible to distinguish between patients with a positive and negative response to BD both at home and at the doctor's office. Therefore, a >12.5% increase in VAS values after BD could indicate that the patient should attend a specialized center for further assessment. In fact, many primary care centers do not have a spirometer or staff trained in the proper use of the device and interpretation of results.

Our study is limited by its cross-sectional nature and the fact that it was conducted in a real-life setting. The number of patients was also low. Therefore, further studies addressing these issues should be conducted.

In conclusion, the present study demonstrated that assessment of the perception of breathing using the VAS could be an initial step in the assessment of the response to BD in adult patients with asthma at home or in the doctor's office.

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*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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## The Addition of Benzylpenicillin Does Not Increase the Skin Test Sensitivity Obtained With Classic ß-Lactam Determinants

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The high prevalence of β-lactam allergy, together with the diversity of this family of antibiotics, all of which are capable of inducing allergic reactions, is an issue of worldwide concern [1,2]. It is essential to improve the accuracy of diagnostic methods, since most patients could be proven tolerant to β-lactams if appropriately evaluated [1]. Guidelines recommend skin testing with the major determinant of benzylpenicillin (BP), penicilloyl-polylysine (PPL), and the minor determinant mixture (MDM). In addition, the European Academy of Allergy and Clinical Immunology (EAACI) has proposed skin testing with amoxicillin (AX) and the culprit β-lactam, when this was neither BP nor AX [2]. In addition, owing to the increased prescription of the combination of AX with clavulanic acid (CLV) and the increasing frequency of selective reactions to this compound [3], it is recommended to use CLV for diagnosis.

Interest in skin testing using BP has recently grown, nourished by the limited availability of the main reagents (PPL and MDM) and the removal of the BP component from the MDM. Indeed, MDM, which was originally composed of BP, the hydrolysis product of BP (benzylpenicilloate), and its decarboxylated product (benzylpenilloate) was recently substituted in Europe by a purer and more stable product composed exclusively of benzylpenilloate (named DM) in order to comply with new regulations [4].

In previous studies, skin testing with BP appeared to improve sensitivity for the diagnosis of penicillin allergy [5,6]. However, the positive predictive value of this approach determined using a drug provocation test has not been established. The aim of the present study was to determine the diagnostic value of skin testing with BP in patients with a consistent clinical history of β-lactam allergy. We performed a prospective study of 97 consecutive patients evaluated for a history of β-lactam allergy (March-October 2014). Diagnosis was based on general EAACI guidelines [2], and patients were classified as immediate reactors (<1 hour) or nonimmediate reactors (>1 hour) and as selective reactors (responding to 1 β-lactam, tolerating BP and/or AX) or cross-reactors (responding to BP). The institutional review boards approved the study, and patients gave their informed consent to participate.

Skin testing was performed using the prick method; if the result was negative, intradermal testing was performed [2] using PPL $1.08 \times 10^{-2}$ mM, DM 1.6 mM, AX 20 mg/mL, CLV 20 mg/mL (Diater-Ferrer), and cephalosporins (CEP) 2 mg/mL. Skin testing with BP (Normon) at 10 000 IU/mL was performed in all patients and in 20 nonallergic controls with proven tolerance to BLs. ImmunoCAP (IDD Thermo Fisher Scientific) with BP and AX and the basophil activation test with BP, AX, CLV, and CEP were performed as described elsewhere [7]. Single-blind placebo-controlled drug provocation testing was performed using BP, penicillin V (PV), AX, AX-CLV, and/or CEP, as previously reported [2]. Regardless of the result of skin testing with BP, all cases with negative results to PPL and DM underwent a drug provocation test with BP (600 000 U). If the result was negative, the patient received a 2-day course of outpatient therapy with PV.

Of the 97 patients included, 23 were confirmed as allergic (23.7%) (Table). The median time between the last reaction and the study was 9.5 months, and the culprit drugs were AX-CLV in 15 cases, AX in 3, CEP in 2, BP in 1, and an undetermined BL in 2. Twenty-two patients experienced an immediate reaction presenting mainly as anaphylaxis (59.1%) or urticaria (36.4%). Among the immediate reactors, 12 had positive skin test results (6 AX, 2 CLV, 2 DM, 1 PPL, and 1 CEP), 9 had a positive basophil activation test result (5 CLV, 2 AX and 2 CEP), 8 had a positive drug provocation test result (4 BP and 4 AX), and 1 had a positive ImmunoCAP result to AX. Only 1 patient had a nonimmediate reaction (maculopapular exanthema 24 hours after administration of AX-CLV), which was diagnosed by a positive delayed intradermal test reading with AX. The diagnoses were cross-reactive BL allergy (7 patients [30.4%]), selective allergy to AX (9 patients [39.1%]), selective allergy to CLV (5 patients [21.7%]), and selective allergy to CEP (2 patients [8.7%]).

Skin testing with BP was positive in 2 patients, both of whom tolerated administration of BP and PV in the drug provocation test. Of these, 1 was finally considered nonallergic and the other (Table, patient 6) was an immediate selective reactor to CLV. These results indicate that skin testing with BP can induce false-positive results in patients with a history of BL allergy.

In the group of patients with cross-reactions and, therefore, allergic to BP (n=7), the sensitivity was 0.0%, as no patient was diagnosed based on a positive result in skin testing with BP. The specificity obtained from the control group was 100%. The positive predictive value and negative predictive value of skin testing were 0.0% and 92.6%, respectively.

In this prospective study, we provide a reliable estimation of the value of skin testing with BP in patients with BP allergy (cross-reactive group) in a population where AX and AX-CLV were the drugs involved in 78.3% of the confirmed reactions. Although the 2 cases with positive results in skin testing with BP found here were similar to those reported elsewhere [5], we found that these patients tolerated BP

Table. Clinical Characteristics and Diagnostic Test Results for Patients With Confirmed Allergy

| Patient | Age, y | Culprit Drug | Initial Reaction | Interval Reaction-Event, mo | Type | Skin Test | ImmunoCAP | BAT | DPT | Allergic Diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 57 | AX-CLV | Urticaria | 3 | Immediate | (+) MDM | (–) | (–) | ND | CR |
| 2 | 18 | AX-CLV | MPE | 10 | Nonimmediate | (+) AX | ND | ND | (–) BP | SAX |
| 3 | 40 | AX | Anaphylaxis | 120 | Immediate | (–) | (–) | (–) | (–) BP (+) AX | SAX |
| 4 | 55 | AX-CLV | Urticaria | 9 | Immediate | (–) | (–) | (–) | (–) BP (+) AX | SAX |
| 5 | 57 | AX-CLV | Anaphylaxis | 24 | Immediate | (+) CLV | (–) | (+) CLV | (–) BP (–) AX | SCLV |
| 6 | 22 | AX-CLV | Anaphylaxis | 1 | Immediate | (+) BP | (–) | (+) CLV | (–) BP (–) AX | SCLV |
| 7 | 38 | BL | Urticaria | 60 | Immediate | (–) | (–) | (+) CLV | (–) BP (–) AX | SCLV |
| 8 | 26 | AX-CLV | Urticaria | 3 | Immediate | (–) | (–) | (+) CLV | (–) BP (–) AX | SCLV |
| 9 | 47 | AX-CLV | Anaphylaxis | 1 | Immediate | (+) AX | (–) | (+) AX | (+) BP | CR |
| 10 | 67 | AX | Anaphylaxis | 60 | Immediate | (+) PPL | (–) | (–) | ND | CR |
| 11 | 66 | BL | UD | UD | Immediate | (+) MDM | (–) | (–) | ND | CR |
| 12 | 61 | AX-CLV | Urticaria | 1 | Immediate | (–) | (–) | (–) | (+) BP | CR |
| 13 | 52 | AX-CLV | Anaphylaxis | 0.25 | Immediate | (–) | (–) | (+) AX | (+) BP | CR |
| 14 | 45 | AX-CLV | Anaphylaxis | 1 | Immediate | (+) AX | (–) | (–) | (–) BP | SAX |
| 15 | 69 | AX-CLV | Urticaria | 264 | Immediate | (+) AX | (–) | (–) | (–) BP | SAX |
| 16 | 65 | BP | Urticaria | 168 | Immediate | (–) | (–) | (–) | (+) BP | CR |
| 17 | 19 | AX-CLV | Anaphylaxis | 12 | Immediate | (+) CLV | (–) | (+) CLV | (–) BP (–) AX | SCLV |
| 18 | 52 | AX-CLV | Anaphylaxis | 24 | Immediate | (–) | (–) | (–) | (–) BP (+) AX | SAX |
| 19 | 35 | CEP | Anaphylaxis | 406 | Immediate | (–) | (–) | (+) CEP | (–) BP (–) AX | SCEP |
| 20 | 32 | CEP | Anaphylaxis | 2 | Immediate | (+) CEP | (–) | (+) CEP | (–) BP (–) AX | SCEP |
| 21 | 50 | AX-CLV | Anaphylaxis | 6 | Immediate | (+) AX | (–) | (–) | (–) BP | SAX |
| 22 | 18 | AX-CLV | Anaphylaxis | 12 | Immediate | (–) | (–) | (–) | (–) BP (+) AX | SAX |
| 23 | 31 | AX | Urticaria | 8 | Immediate | (+) AX | (+) AX | (–) | (–) BP | SAX |

Abbreviations: AX, amoxicillin; BAT, basophil activation test; BL, ß-lactams; BP, benzylpenicillin; CEP, cephalosporin; CLV, clavulanic acid; CR, cross-reactive; DPT, drug provocation test; MDM, minor determinant mixture; MPE, maculopapular exanthema; ND, not done; PPL, penicilloyl-polylysine; SAX, selective amoxicillin; SCEP, selective cephalosporins; SCLV, selective clavulanic acid; UD, undetermined.

and PV. This false-positive rate should be highlighted, since the results could lead to avoidance of penicillin, with the subsequent costs and potential side effects that result from the use of other non–β-lactam antibiotics [1]. Avoiding unnecessary treatment with β-lactams impacts patient morbidity by increasing the risk of multiresistant bacteria [8] and increases health care costs. This approach has been estimated to cost Can $326.50 per patient in a hospital setting when antibiotics other than standard-of-care penicillin are used [9]. Moreover, as the negative predictive value of skin testing with BP (92.6%) is lower than that of PPL (99%) [10], the usefulness of this approach should be reconsidered. It is important to remember that skin testing with all the reagents used in this study only confirmed the diagnosis in 47.8% of cases; in the remaining 52.2%, the basophil activation test, ImmunoCAP, or drug provocation testing was necessary, thus emphasizing the need to use a full assessment to confirm β-lactam allergy.

To conclude, we found no clear benefits of adding skin testing with BP to a diagnostic algorithm that already included

PPL and MD for a population where AX/AX-CLV were the main drugs involved in reactions.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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## Potentially Inappropriate Prescription in Patients With a History of Allergy to ß-Lactam Antibiotics: A Health Care Challenge

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ß-Lactam allergy is the most widely reported type of drug hypersensitivity [1]. However, 80%-95% of patients with suspected ß-lactam allergy have a negative result in diagnostic tests and, therefore, cannot be considered allergic [2]. Furthermore, the frequency of cross-reactivity between ß-lactams is relatively low (approximately 10%), while allergies to specific drugs are frequently diagnosed [3].

Patients with suspected ß-lactam allergy are often treated with broad-spectrum antibiotics such as vancomycin and fluoroquinolones, leading to unnecessary overprescription of these drugs. Overprescription in turn leads to an increase in the emergence of resistant strains [4], such as multiresistant *Pseudomonas* species, owing to the use of fluoroquinolones, or outbreaks of vancomycin-resistant *Enterococcus* species and methicillin-resistant *Staphylococcus aureus* [5]. Overprescription of second-line antibiotics is also risky (nephrotoxicity due to vancomycin or colitis induced by *Clostridium difficile* related to the use of fluoroquinolones) and expensive.

Despite the availability of validated diagnostic tests, many patients with suspected ß-lactam allergy are classified as allergic without undergoing diagnostic tests [6].

According to international recommendations for the study of ß-lactam allergy [7], skin tests are the primary diagnostic tool, complemented with in vitro tests such as the measurement of total and specific IgE and the basophil activation test. Furthermore, drug provocation testing is still necessary to reach a definitive diagnosis [8].

The aims of our study were as follows: (*1*) to confirm the real prevalence of ß-lactam allergy in our setting; (*2*) to quantify overprescription of second-line antibiotics in patients with a history of ß-lactam allergy; and (*3*) to assess the cost of replacing ß-lactams with second-line antibiotics.

The study was conducted in 2 stages:

(*1*) Retrospective observational study in a tertiary hospital. Medical records of adult patients admitted during 2012 who had been prescribed levofloxacin and/or vancomycin (main alternatives in patients with ß-lactam allergy) were reviewed. Moreover, we verified whether the prescription was a consequence of the suspected allergy or was indicated for the patient's condition. Patient data were reviewed and screened using the institutional electronic prescribing system (SAP Medication).

(*2*) In order to calculate the actual prevalence of allergy in our setting, the Allergy Unit conducted a protocol-based allergy study [7] in a representative sample of patients with suspected ß-lactam allergy.

The assessment of the impact on costs (€/patient/day) due to the overuse of second-line antibiotics was calculated by taking into account the direct costs of the standard treatment (according to our institutional therapeutic protocols) and its alternative.

We reviewed 3668 medical records of inpatients admitted during 2012 who received levofloxacin and/or vancomycin. ß-Lactam allergy was reported in 10.5% (n=368), although allergy was confirmed in the clinical history of only 3.26% of patients. Among these patients, 55% (n=212) were prescribed a second-line antibiotic as a consequence of the suspected allergy, while in the remainder, prescription was indicated for the patient's condition according to our hospital therapeutic protocols.

Among patients with suspected allergy (368 patients), a representative sample of 26 (calculated using the Wilson method, assuming a prevalence of 5%-10%, a confidence interval of 95% and an accuracy of 10%) was selected for the protocol-based study. The clinical history was taken, and blood sampling and the study were performed in the Allergy Unit.

Of the 26 patients, only 2 (7.7%; 95%CI, 6.2%-20.6%) had a positive diagnosis of ß-lactam allergy; both patients were allergic to amoxicillin. Therefore, the real prevalence of ß-lactam allergy calculated in the total sample of patients (n=3668) was 0.7%-2.1%.

Furthermore, potentially inappropriate prescription of second-line antibiotics, considering the upper limit of the confidence interval (20.6%), was close to 80%. In other words, 170/212 patients were prescribed a broad-spectrum antibiotic such as vancomycin and levofloxacin when a ß-lactam could have been used as first-line treatment.

We also found that diagnostic tests are rarely used in our setting (3.26% of all patients with suspected allergy). The Figure shows the distribution of patients in the study.

As for cost, which was calculated in 191/212 patients (conditions with a defined standard therapy in our setting), the use of broad-spectrum antibiotics instead of first-line drugs led to a median increase of 49.3% in the cost of treatment (difference of €9.95/patient/day), which is consistent with results from other, similar studies [9]. However, our calculation was based only on direct costs and did not take into account costs derived from possible antimicrobial resistance or therapeutic drug monitoring.

Even though the emergence of multidrug-resistant bacteria is becoming a serious health problem, few studies have measured the real percentage of patients with allergy to
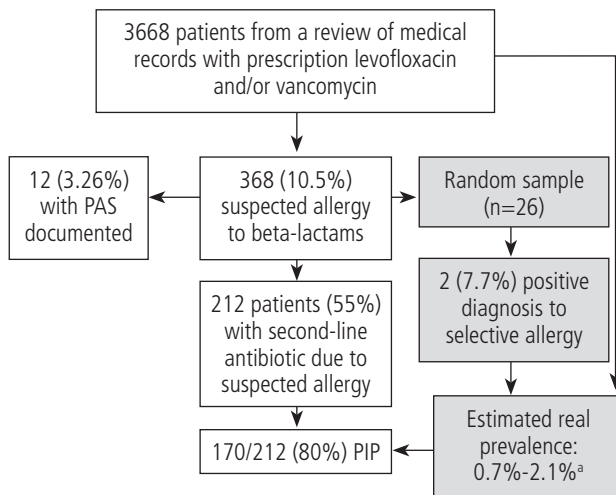
**Figure.** Distribution of Patients in the Study. PAS indicates protocolized allergy study; PIP, potential inappropriate prescription (ie, patient treated with vancomycin and/or levofloxacin who could have been treated with a ß-lactam).
[a]Calculated by extrapolation of the lower and upper and limit of the 95% confidence interval (6.2%-20.6%).

ß-lactam antibiotics. In this sense, our study provides evidence from a specific setting. The study was limited to the evaluation of patients who were prescribed vancomycin and levofloxacin, because these are the main therapeutic alternative in ß-lactam allergy; consequently, the total number of allergic patients might be higher. In addition, the protocol-based study was performed in a representative sample of patients and not in the entire population with a history of allergy.

Despite these limitations, the percentage of ß-lactam allergy found (both real and estimated) is consistent with the prevalence rates published by other authors.

Our findings confirm that the real prevalence of ß-lactam allergy among hospitalized patients is low, while the unnecessary prescription of broad-spectrum antibiotics in patients with suspected allergy is very high.

Park et al [10] performed a prospective study with the aim of assessing collaboration between allergists and pharmacists to reduce overprescription and concluded that interdisciplinary collaboration improves patient safety and optimizes antibiotic prescription. Similarly, Wall et al [6] found that the skin tests recommended by hospital pharmacists in patients with a history of ß-lactam allergy can help to prevent inappropriate use of second-line antibiotics.

Therefore, our findings and the serious consequences of overprescription of second-line antibiotics indicate that multidisciplinary collaboration should be promoted to increase safe ß-lactam prescription.

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*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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## Genetic Analysis as a Practical Tool for Diagnosis of Hereditary Angioedema With Normal C1 Inhibitor: A Case Report

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Key words: Hereditary angioedema. Hereditary angioedema with normal C1 inhibitor. Bradykinin. Coagulation factor XII. *F12* gene.

Palabras clave: Angioedema hereditario. Angioedema hereditario con C1 inhibidor normal. Bradiquinina. Factor de coagulación XII. Gen *F12*.

Hereditary angioedema (HAE) is an autosomal dominant disease characterized by episodes of edema of the face, extremities, and larynx and recurrent attacks of severe abdominal pain due to edema of the intestinal mucosa [1]. Classic HAE is caused by deficiency of C1 inhibitor (C1-INH), leading to increased activation of the kallikrein-kinin pathway, with overproduction of bradykinin, which increases vascular permeability and induces angioedema [1]. Mutations in the gene coding for coagulation factor XII (*F12*) have been identified in a subset of patients who present a dominantly inherited disease with features similar to those of patients with HAE due to C1-INH deficiency (C1-INH-HAE) [2]. These patients have no abnormalities in complement parameters and no mutations in *SERPING1*, the gene coding for C1-INH [2]. Symptoms are often worse in women taking estrogen-containing medications and pregnant women in C1-INH-HAE as well as in HAE with normal C1-INH [2]. We report the case of a patient with HAE and normal C1-INH and no family history of angioedema who remained undiagnosed for 19 years.

The patient was a 35-year-old woman with recurrent episodes of abdominal pain that started at the age of 16 years. The episodes were of mild to moderate intensity, although during the past 5 years, they had become severe and were associated with vomiting and diarrhea. The patient had to attend the emergency department on several occasions and required intravenous medication. On 2 occasions, abdominal computed tomography revealed ascites and edema of the intestinal wall (Figure, A and B). Despite being evaluated by several gastroenterologists and undergoing a comprehensive workup, the patient remained undiagnosed, and the abdominal pain attacks were attributed to irritable bowel syndrome. During these attacks, her blood tests showed normal amylase and lipase levels, elevated white cell counts, and elevated C-reactive protein concentrations. No eosinophilia, anemia, or other abnormalities were present. Endoscopy revealed esophagitis involving the distal esophagus and mild pangastritis; colonoscopy findings were normal. During the previous 5 years, she had also experienced episodes of edema of the lips and face lasting 48 to 72 hours, despite treatment with intravenous corticosteroids and antihistamines (Figure, C and D). She had received many prolonged courses of oral corticosteroids and continuous treatment with antihistamines, which did not prevent attacks. She had 1 episode of tongue edema, but no respiratory discomfort during the attacks.
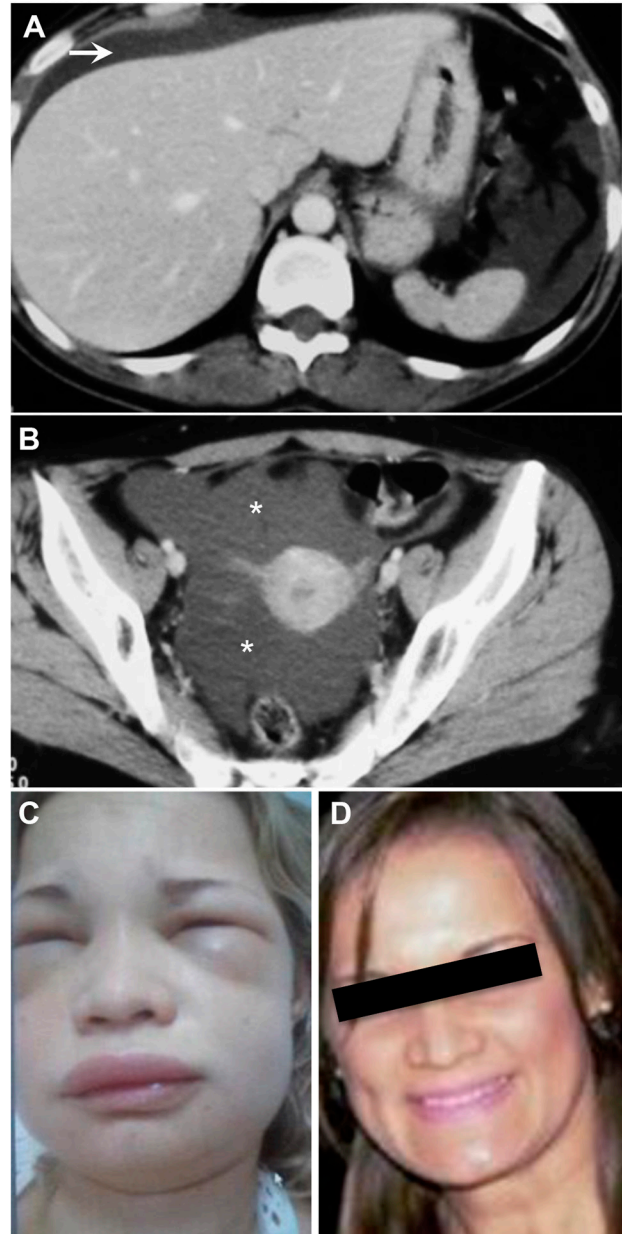


**Figure.** Manifestations of HAE with normal C1-INH. A, Computerized tomography scan showing ascites (arrow). B, Large amount of fluid (*) in the pelvis. C, Angioedema of lips and face. D, Patient during attack-free period.

At the time she was attending our Allergy Clinic, she experienced episodes of abdominal pain and/or facial or lip edema every 2 weeks that required constant visits to the emergency department, with a considerable impact on her quality of life. When asked about use of oral contraceptives, she acknowledged that worsening of symptoms showed a relationship with the start of a combined estrogen/progesterone contraceptive. Her medical history revealed asthma during childhood, and current mild symptoms of allergic rhinitis. She was prescribed pantoprazole and pinaverium bromide by her gastroenterologist, with no improvement in the abdominal symptoms.

We considered a diagnosis of HAE very likely, discontinued the estrogen-containing contraceptive, and switched her contraception to a progestogen-only pill (desogestrel). This measure resulted in complete remission of symptoms (none during the past 12 months). C1-INH and C4 levels were normal, and C1-INH activity in the intervals between attacks was 109% by the chromogenic method (reference value, 70%-130%). A genetic analysis was performed based on allelic discrimination and sequencing of exon 9 and the adjacent intronic regions of *F12*, and the patient was found to be positive for the Thr328Lys mutation. Three of her family members were also found to carry Thr328Lys: her father and her 40-year-old sister were asymptomatic for angioedema and/or abdominal attacks, although her 24-year-old nephew had presented episodes of abdominal pain of mild to moderate intensity since childhood.

It is not unusual for patients with HAE to remain undiagnosed for several years after onset of symptoms [3]. The findings in the present case lead us to recommend the inclusion of HAE in the diagnosis of recurrent abdominal pain, which can be the initial clinical presentation in patients with HAE [4,5], and to point out the difficulties in diagnosing patients with HAE with normal C1-INH. According to a recently updated classification, the patient we report fulfilled the criteria for factor XII-HAE (FXII-HAE) [6]. This term has been recommended not only when there is family history of angioedema, but also in the absence of a family history. However, a major limitation of identifying *F12* mutations as a routine diagnostic test is the fact that mutations are not present in all cases of HAE with normal C1-INH [7,8]. In the absence of an *F12* mutation, the diagnostic possibilities would include HAE of unknown origin (U-HAE), when a family history is present but markers of genetic disease are absent, and idiopathic nonhistaminergic acquired angioedema (InH-AAE), which is characterized by the absence of a family history and of an underlying genetic defect [6]. However, a U-HAE patient could found an HAE family, and a lack of family history does not rule out this possibility, thus hampering confirmation of diagnosis. Although the pathogenic mechanisms for FXII-HAE, U-HAE, and InH-AAE are not fully understood, evaluation of patients categorized as HAE or AAE of unknown origin revealed that those with HAE experience earlier onset of symptoms and a higher frequency of laryngeal and abdominal attacks, whereas patients with acquired forms more often present angioedema of the face and oral mucosa, suggesting that these conditions may be intrinsically different [6].

In Brazil, we detected a higher frequency of the *F12* mutation in patients with HAE with normal C1-INH than previously reported (20%-30%) [7]. The Thr328Lys mutation was found in 17/40 (42.5%) nonrelated families with HAE and normal C1-INH levels and activity. Five of these families were previously described [8], including 4 with at least 1 member carrying the Thr328Lys mutation.

At individual patient level, definitive diagnosis is important for advice on treatment of acute attacks and prophylaxis, genetic counseling, and preventive measures [9]. No controlled studies have addressed treatment of patients with HAE and normal C1-INH, although case reports have shown success in treating acute attacks of angioedema with C1-INH concentrate, icatibant, ecallantide, progesterone, danazol, and tranexamic acid, which are used in classic HAE, thus reinforcing the likely role of bradykinin in the pathogenesis of HAE with normal C1-INH [2,10].

*Conflicts of Interest*

The authors declare that they have no conflict of interest.

*Previous Presentation*

The data included in this manuscript were presented in poster format during the Annual Meeting of the American Academy of Allergy, Asthma and Immunology in Houston, Texas, February 6-9, 2015 (Arruda LK et al, Genetic analysis as a practical tool to diagnose hereditary angioedema with normal C1 inhibitor: a case report. J Allergy Clin Immunol. 2015; 135(2):AB197).

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# Does the Effect of Smoking on Fractional Exhaled Nitric Oxide Vary by Race in Adults in a US National Survey?

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Asthma and chronic obstructive pulmonary disease are important causes of mortality and morbidity in adults [1,2]. Inflammation of airways is an important mechanism leading to airway obstruction in these common conditions [3]. Asthma mortality and morbidity are higher in black than white adults; some asthmatics are smokers. In atopic asthma, inducible nitric oxide (NO) synthetase is upregulated in respiratory epithelium and produces increased NO concentrations in exhaled air [4]. Alvarez-Puebla et al [5] have reported on considerations in interpreting fractional exhaled nitric oxide (FeNO), which has gained favor in identifying and monitoring eosinophilic airway inflammation in patients with asthma or chronic obstructive pulmonary disease being considered for inhaled corticosteroid therapy [5]. Little information is available on the racial differences in the well-known lowering effect of cigarette smoking on FeNO [4]. To estimate the difference in association between current smoking and FeNO between non-Hispanic black and white US adults, this study examined data from a large, US national sample.

The National Health and Nutrition Examination Survey (NHANES) is designed to assess the health and nutritional status of adults and children in the United States [6,7]. Response rates for 2-year study cycles exceed 75%. All participants/agents provided written informed consent. Serum cotinine was analyzed using an isotope dilution liquid chromatography tandem mass spectrometry method. Participants were categorized as not smoking if the serum cotinine level was less than 10.0 ng/mL and currently smoking if it was 10.0 ng/mL or greater. FeNO was measured using the Aerocrine NIOX MINO, a portable, handheld NO analyzer (Aerocrine AB). This analysis uses the average of 2 reproducible measurements [6,7] A 2-sided *t* test was used to compare the means with 95% CIs of ln FeNO values. Multivariable linear regression modeling using Stata Version 11 assessed smoke exposure by history as a predictor of ln FeNO. Data analyses using Stata Version 11 accounted for the complex survey design.

Between 2007 and 2012, NHANES interviewed 30 442 participants and 29 353 underwent examination. Of these 17

404 were aged 20 and over, and 13 207 had 2 reproducible FeNO measurements. Those 12 557 who also had complete data on demographics and serum cotinine levels formed the analytic sample. Mean ln FeNO among non-Hispanic whites was 2.698 in nonsmokers and 2.132 (difference, 0.565) in smokers with no history of asthma. Mean ln FeNO among non-Hispanic blacks was 2.715 in nonsmokers and 2.232 (difference, 0.483) in smokers with no history of asthma. In weighted linear regression analyses controlling for age, gender, and asthma history, the interaction term for smoking and black race was not significant ($P$=.357). This finding was unchanged in sensitivity analyses excluding those with a history of asthma, smoking within 1 hour of testing, and controlling for consuming NO-rich foods before the test, or a respiratory infection in the week before the test.

The current study found that cigarette smoking was associated with lower FeNO compared with not smoking. However, the strength of this association did not differ between black and white non-Hispanics. The mechanism by which tobacco smoke exposure lowers FeNO may be that it increases arginase activity and therefore decreases arginine availability for NO production in the airways [8]. Others have suggested negative feedback of NO in smoke on inducible NO synthase [9]. A polymorphism in the NO synthase and related genes may modify the effect of smoking [9,10]. Strengths of the study include the large, representative sample of the US population, oversampling of blacks, and standardized measurement protocols with quality control checks. Limitations include lack of data on some possible confounders, such as recent exposure to ozone air pollution. In conclusion, a model for mean ln FeNO controlling for age, gender, and asthma history testing the interaction of cigarette smoking with race did not provide evidence for significant variation of the effect of smoking on FeNO between blacks and whites.

*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

*Previous Presentation*

The content of this paper was presented at the National Health Statistics Conference in, Bethesda, Maryland, United States on August 25, 2015.

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# Home-Based Oral Immunotherapy With a Baked Egg Protocol

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The increasing prevalence of food allergy means that allergen avoidance alone is no longer a viable management approach. There is an increasing need for widely available disease-modifying interventions [1]. Oral immunotherapy (OI) is one such approach, with promising results in milk, egg, and peanut allergy [2].

Egg allergy is common in young children and has been shown to be more prevalent and persistent than previously thought [3]. OI to hen's egg has been administered using raw egg [4], powdered or pasteurised egg white [5], dehydrated egg white [6], and lyophilized egg protein [7]. Despite allergenic differences between these preparations (processing and cooking alter the allergenicity of egg), the various forms enabled over 80% of patients to be successfully desensitized. However, these methods are more suited to research settings and are consequently not widely available in clinical practice. In addition, very low doses and specific flavorings or vehicles were required to enhance palatability [5].

Baked egg and cooked egg provide a more practical and palatable option, although their efficacy for developing tolerance to more allergenic forms of egg is uncertain [8]. Cooked egg has been used in OI either alone [9] or after powdered egg, which was deemed unsuitable for large daily doses over long periods [10]. Baked egg has not yet been used in OI. It is possibly more palatable to egg-allergic children, who generally do not like the texture or taste of whole egg, and more practical than whole egg, as it is eaten in biscuits or cakes and can therefore be stored for longer and served in small doses.

We developed a recipe for an egg-containing biscuit to be used in baked egg OI as proof of concept and report on its efficacy and safety in home-based, slow updosing OI in children with persistent egg allergy.

We made square biscuits measuring about 1 cm thick and weighing 100 g, which were then baked at 150°C for 15 minutes and cut into squares measuring 1 cm × 1 cm. Each square weighed about 1 g. The recipe required 5 stages to allow for gradual increases in the amount of egg ingested at each successive dose without having to eat too large a portion of biscuit. The ingredients were plain flour (40 g at stages 1-4 and 80 g at stage 5), sugar (40 g at all stages), margarine (25 g at stages 1-3, 15 g at stage 4, and none at stage 5), and egg (0.1 mL at stage 1, 0.5 mL at stage 2, 1.0 mL at stage 3, 10 mL at stage 4, and 50 mL at stage 5).

The first session was supervised in hospital with subsequent updosing at home. The initial dose was equivalent to 125 μg of egg protein, which was increased daily over 60 days to a target maximum dose of 6.25 g of egg protein (Table). Once the maximum dose was achieved, patients underwent an open challenge with boiled egg to assess whether whole egg was also tolerated.

Fifteen children with IgE-mediated egg allergy (9 boys; median age, 11 years and 2 months; range, 6-17 years) were recruited at routine clinical appointments. All 15 children fulfilled the inclusion criteria of age >5 years and persistent IgE-mediated egg allergy with a positive skin prick test (ALK Abelló) result to egg white and egg yolk (>3 mm) and symptoms after eating baked egg evidenced by an allergic reaction in the previous 6 months or a positive open food challenge result. Additional atopic conditions reported included other food allergies (12 patients), allergic rhinitis (11 patients), eczema (9 patients), and asthma (4 patients). The worst previous symptoms with egg were predominantly oral (itchy mouth or throat), cutaneous (urticaria and angioedema), and gastrointestinal (abdominal pain, nausea, and vomiting). Only 1 patient experienced upper airway symptoms (throat tightness), and 1 experienced lower airway symptoms (cough and wheeze).

Eight of the 15 patients (53%) completed the OI program: 4 in the protocol target of 60 days, as they had no adverse effects or allergic symptoms, and 4 between 80 and 270 days. Of the remaining 7 patients, 2 could not tolerate the first dose without symptoms and thus remained on strict egg-free diets, and 5 achieved partial tolerance at days 10 to 47, allowing them to include previously avoided trace amounts of egg into their diets. All adverse reactions were mild and could be controlled by oral antihistamines where needed.

OI provides protection against egg allergy despite variations in the form of egg, although protocols based on uncommon forms (egg powder, liquid raw egg) are not suitable for clinical use, and rapid updosing regimens are more commonly associated with allergic adverse reactions [10]. We sought to develop a therapy for egg allergy that would be safe and effective and immediately and widely available to patients in our clinical setting.

Baked egg (ie, egg cooked with wheat) has not been used as an updosing preparation in egg OI. If effective as an OI preparation, it offers potential advantages. Perhaps the most obvious advantage is that the baked egg–allergic patient, who was traditionally instructed to avoid all forms of egg in his or her diet, is now instructed to do exactly the opposite. Eating eggs therefore in a form that they are most likely to encounter provides visual reassurance, where OI is successful, that the food is safe. Furthermore, as baked egg is a less allergenic form of egg, it is possible that a greater percentage of patients who have to avoid all types of egg will be successfully treated. Whilst baked egg tolerance does not provide protection against raw egg, regular ingestion of baked egg accelerates the resolution of egg allergy and can lead to tolerance of less well-processed and more allergenic

Table. Protocol for oral immunotherapy up dosing with baked egg biscuits

| Egg Volume[a] | Day | No. of Biscuit Squares[b] | Amount of Egg Protein | Egg Volume | Day | No. of Biscuit Squares | Amount of Egg Protein |
|---|---|---|---|---|---|---|---|
| | | Stage 1[c] | | | | Stage 4 | |
| 0.1 mL | 1 | 1 | 125 (µg) | 10 mL | 32 | 2 | 25.00 (mg) |
| | 2 | 2 | 250 | | 33 | 3 | 37.50 |
| | 3 | 3 | 375 | | 34 | 4 | 50.00 |
| | 4 | 4 | 500 | | 35 | 6 | 75.00 |
| | 5 | 5 | 625 | | 36 | 8 | 100.00 |
| | 6 | 6 | 750 | | 37 | 10 | 125.00 |
| | 7 | 7 | 875 | | 38 | 12 | 150.00 |
| | 8 | 8 | 1000 | | 39 | 14 | 175.00 |
| | 9 | 9 | 1125 | | 40 | 16 | 200.00 |
| | | | | | 41 | 20 | 250.00 |
| | | Stage 2 | | | 42 | ¼ biscuit | 312.50 |
| 0.5 mL | 10 | 2 | 1.250 (mg) | | 43 | 30 | 375.00 |
| | 11 | 3 | 1.875 | | 44 | 35 | 437.50 |
| | 12 | 4 | 2.500 | | 45 | 40 | 500.00 |
| | 13 | 5 | 3.125 | | 46 | ½ biscuit | 625.00 |
| | 14 | 6 | 3.750 | | | | |
| | 15 | 7 | 4.325 | | | Stage 5 (Fraction of biscuit)[d] | |
| | 16 | 8 | 5.000 | | | | |
| | 17 | 9 | 5.625 | 50 mL[d] | 47 | 2/16 | 780.00 |
| | 18 | 10 | 6.250 | | 48 | 3/16 | 1.17 (g) |
| | | | | | 49 | ¼ biscuit | 1.56 |
| | | Stage 3 | | | 50 | 5/16 | 1.95 |
| 1.0 mL | 19 | 6 | 7.500 | | 51 | 6/16 | 2.34 |
| | 20 | 7 | 8.750 | | 52 | 7/16 | 2.73 |
| | 21 | 8 | 10.00 | | 53 | ½ biscuit | 3.12 |
| | 22 | 9 | 12.50 | | 54 | 9/16 | 3.51 |
| | 23 | 10 | 13.75 | | 55 | 10/16 | 3.56 |
| | 24 | 11 | 15.00 | | 56 | 11/16 | 4.29 |
| | 25 | 12 | 16.25 | | 57 | ¾ biscuit | 4.68 |
| | 26 | 13 | 17.50 | | 58 | 13/16 | 5.07 |
| | 27 | 14 | 18.75 | | 59 | 14/16 | 5.46 |
| | 28 | 15 | 20.00 | | 60 | Full biscuit | 6.25 |
| | 29 | 16 | 21.25 | | | | |
| | 30 | 17 | 22.50 | | | | |
| | 31 | 18 | 23.75 | | | | |

[a]Volume of egg in stage recipe.
[b]Biscuit in stages 1 to 4 cut into squares measuring 1 cm x 1 cm..
[c]One biscuit for stages 1 and 2, 2 biscuits for stage 3, 8 for stage 4, and 16 for stage 5.
[d]Biscuit in stage cut into quarters, then half, and then half again to 1/16 pieces.

forms of egg [1]. Indeed, such was our experience, as, at the end of our baked egg updosing protocol, all patients who completed the protocol were also able to eat—and were therefore tolerant to—whole egg.

*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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# Different Clinical Phenotypes in 2 Siblings With X-Linked Severe Combined Immunodeficiency

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Severe combined immunodeficiency (SCID) is a heterogeneous group of disorders characterized by profound impairment of cellular and humoral immunity [1]. X-linked SCID (XSCID) is the most common form of SCID and is caused by mutations in *IL2RG*, the gene encoding the common γ chain (γc) shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Patients with XSCID are characterized by very low numbers of circulating T and NK cells and an increased proportion of B cells. Although maternal T-cell engraftment is the most common reason for the presence of circulating T cells in XSCID patients [2], atypical cases of XSCID with mild disease and normal numbers of autologous T and/or NK cells have been reported. Patients were found to carry either specific missense mutations [3-5] or splice-site mutations [6,7] leading to severely reduced levels of functional γc protein, although residual cytokine signaling and development of peripheral T and/or NK cells were also observed. In addition, spontaneous reversion of genetic defects was recently demonstrated to be the basis for the milder immunological phenotypes in patients with primary immunodeficiency [8].

We report on 2 siblings with atypical XSCID who showed different clinical and immunological features. They were born from nonconsanguineous Japanese parents. The clinical and immunological data of patient 1 have been reported elsewhere [9]. Briefly, patient 1 developed a generalized erythematous rash and alopecia at 1 month of age, followed by persistent cough, fever, hepatosplenomegaly, and lymphadenopathy. He was hospitalized because of failure to thrive, protracted diarrhea, and hypoproteinemia at 5 months of age. His clinical and laboratory findings (erythroderma, lymphadenopathy, hepatosplenomegaly, eosinophilia, low serum IgG, elevated serum IgE, and the presence of activated T cells) (Figure, A) were reminiscent of Omenn syndrome.
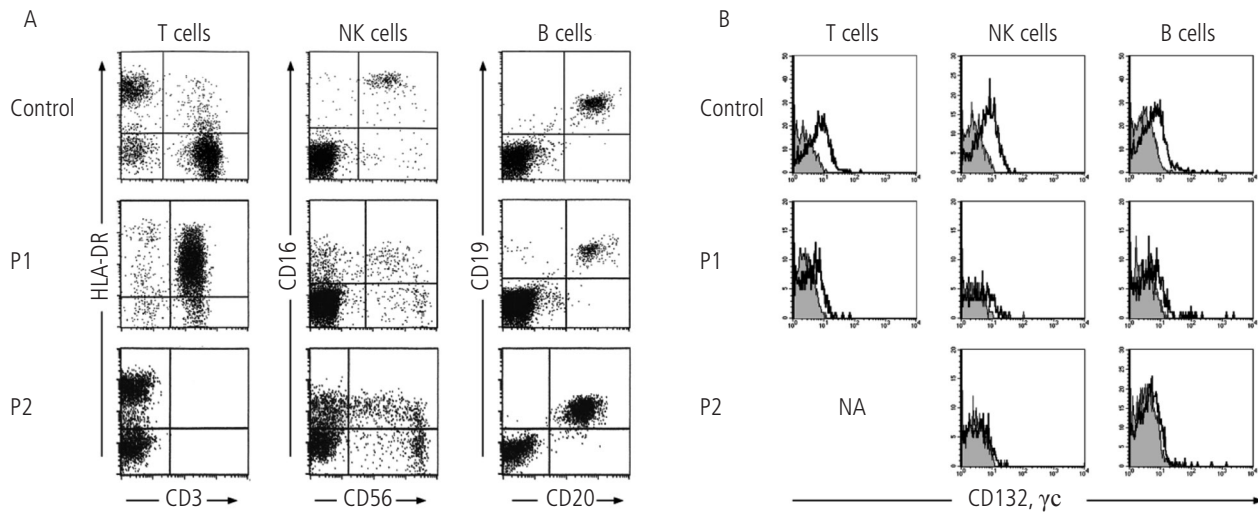
Figure. A, Immunophenotyping of lymphocyte subsets. B, Analysis of γc expression on lymphocyte subsets. Solid peaks indicate control antibody. Open peaks defined by bold lines represent anti-CD132 monoclonal antibody. P1 indicates patient 1; P2, patient 2.

Microsatellite analysis ruled out maternal T-cell engraftment. A thymic shadow was present on the chest radiograph. The patient exhibited revertant T-cell mosaicism caused by a second-site mutation predominately in the skin [9]. Once the diagnosis of atypical XSICD (T+ NK+ B+) was confirmed using molecular techniques, patient 1 underwent cord blood transplantation but died of complications at 10 months of age.

The younger brother of patient 1 (patient 2) was diagnosed with XSCID at birth because of a positive family history and defective γc expression in cord blood lymphocytes. A chest X-ray revealed no thymic shadow. Patient 2 had autologous NK cells but no T cells (T⁻NK⁺B⁺) (Figure, A) and, unlike patient 1, showed no manifestations of Omenn syndrome. He was free from infections on prophylactic antibiotic treatment and underwent successful cord blood transplantation at 46 days of age. He has been in good health with no evidence of disease for 5 years.

Both patients carried the same splice-site mutation at the donor site of intron 1 in the *IL2RG* gene (IVS1+5G>A). This mutation was listed in the *IL2RG* database (http://research. nhgri.nih.gov/apps/scid/IL2RGbase.shtml), and the mother was a heterozygous carrier. The mutation caused most of the mRNA to be incorrectly spliced but produced normally spliced transcripts in lesser amounts [9]. In the incorrectly spliced transcripts, the first 28 nucleotides from intron 1 were incorporated into exon 2 due to a cryptic 5' splice site in intron 1, resulting in a frameshift mutation and premature termination. Accordingly, the lymphocytes showed the residual γc protein that likely enabled T- and/or NK-cell development to occur (Figure, B). Similar results were obtained in another case study of atypical XSICD with poorly functioning peripheral T cells [6]. As in the cases we report here, the patient in question carried a splice site mutation located at the end of exon 1 (129G>A). This mutation caused the same aberrant mRNA splicing, resulting in the generation of both an abundant, nonfunctional isoform containing the first 28 bp of intron 1

using the same cryptic 5' splice site and a normally spliced, functional isoform with a missense mutation (D39N) in limited amounts.

The levels of residual γc expression were similar in B and NK cells from both patients (Figure, B). T cells from patient 1 also showed γc expression at levels comparable to those of his B and NK cells. The reason underlying the appearance of autologous T cells in the older brother only is unclear. Peripheral T cells obtained from patient 1 at 7 months of age showed normal absolute numbers ($3.7 \times 10^9$/L), an activated phenotype, and a moderately restricted T-cell receptor repertoire. However, analysis of T-cell receptor excision circles (TRECs) by real-time polymerase chain reaction revealed undetectable levels of TRECs in peripheral blood from patient 1 (<100 copies/μg DNA; normal range, 3500-8100) [10]. As expected, TRECs were not detected in patient 2 (T⁻NK⁺B⁺ SCID phenotype). These results suggested that the T cells of patient 1 were generated and expanded from a few T-cell progenitors, resulting in a low level of thymic output at 7 months of age.

At 1 month of age, patient 1 developed Omenn syndrome–like manifestations, which might have resulted from both impaired immune tolerance and defective immune regulation. As discussed in our previous report [9], his complicated condition, including the presence of autologous γc-defective T and NK cells in peripheral blood and the expansion of revertant γc-expressing T cells in the skin, does not allow us to draw clear conclusions about which aspects are responsible for the development of the Omenn syndrome phenotype. Whatever causal mechanism underlies this phenotype, it is important to note that patient 2 had no circulating mature T cells or revertant mosaicism and did not develop any Omenn syndrome-like manifestations.

In summary, our study demonstrated that the same γc splice-site mutation caused different leaky phenotypes presenting as either T⁺NK⁺B⁺ or T⁻NK⁺B⁺ SCID within 1

family. Leaky γc mutations may lead to a diverse spectrum of clinical and immunological phenotypes.

*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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## Successful Capecitabine Desensitization for a Delayed-Type Hypersensitivity Reaction

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Fluorouracil (5-FU)-based chemotherapies are used to treat many types of cancer, including advanced colorectal, gastric, and breast cancer. With the development of capecitabine, an oral 5-FU drug, many chemotherapy regimens changed to oral regimens since outpatient treatments are more convenient and cheaper [1,2].

The most common cutaneous adverse effect of capecitabine is hand–foot syndrome, also known as palmar–plantar erythrodysesthesia. Alopecia, nail changes, skin discoloration, cutaneous hyperpigmentation, photosensitivity, and radiation recall reactions have also been reported [2,3]. However, delayed-type hypersensitivity reactions such as maculopapular eruptions have not yet been described.

A 74-year-old man, diagnosed with stage III colon cancer, was prescribed a treatment plan of 6 cycles of the XELOX regimen, which consists of capecitabine (2000 mg/m$^2$ twice daily) in D1-14/q21 day cycles and oxaliplatin (130 mg/m$^2$) as an adjuvant treatment after surgery. The patient experienced generalized pruritus followed by maculopapular eruptions on the trunk and extremities on the second day of capecitabine, 8 hours after the last dose, and was subsequently referred to our outpatient allergy clinic. Laboratory tests including liver and renal function tests, as well as a complete blood count, were normal. Capecitabine was discontinued and the patient was treated with corticosteroids for 5 days and antihistamines for the following 10 days. After the resolution of all clinical symptoms, intradermal tests with oxaliplatin (1/1000, 1/100 and 1/10 dilutions) and patch tests with both oxaliplatin and capecitabine were performed. For the patch tests, the commercialized forms of capecitabine and oxaliplatin were diluted at a concentration of 30% in petrolatum. Results were read 20 minutes after application, as well as on the second and fourth days, as suggested [4]. The delayed readings of the intradermal tests with oxaliplatin and the patch tests were negative. A parenteral drug provocation test with oxaliplatin was performed at 30-minute intervals with increasing doses of 13 mg (1/10 of the total dose), 32.5 mg (1/4), and the remaining dose of 84.5 mg. No reactions were observed during the test or in the following 2 days. As maculopapular eruptions due to capecitabine had not been previously reported, an oral drug provocation test was performed to confirm the diagnosis. A 125-mg dose, corresponding to a quarter of the standard dose, was planned to be given in 4 repeated doses at 3-hour intervals. One hour after the second dose, the patient reported itching all over his body. Although pruritus is a subjective sign, considering the previous pruritic rash experienced by the patient, the test was considered positive and stopped. A 180-mg dose of fexofenadine was given to relieve the symptoms. As capecitabine was considered to be essential for the survival of the patient, a desensitization protocol was prepared. A 2000 mg/m$^2$ daily dose of capecitabine had been recommended for his treatment.

The desensitization protocol began with a 25-mg dose (1/20 of one 500-mg Xeloda tablet), which corresponds to approximately 1/100 of the total dose. As the patient's previous reaction had been a delayed-type hypersensitivity reaction, desensitization was performed using slow incremental doses over 16 days. Each day, 2 doses were given at 3-hour intervals. On the eighth day, a total of 2000 mg was planned to be given in 2 half doses to the patient. However, 2 hours after the first dose of 1000 mg, the patient experienced pruritus without eruptions. Consequently, the second dose was not given and daily antihistamine treatment was initiated. Over the following 6 days, the last tolerated dosage of 1200 mg per day was repeated, and the desensitization was successfully completed in a total of 16 days. The suggested final desensitization protocol is illustrated in the Table.

**Table.** Recommended Capecitabine Desensitization Protocol for Patients With Delayed-Type Hypersensitivy Reactions

| Day | First Dose | Second Dose | Daily Cumulative Dose |
|-----|-----------|------------|----------------------|
| 1 | 25 mg | 50 mg | 75 mg |
| 2 | 75 mg | 75 mg | 150 mg |
| 3 | 150 mg | 150 mg | 300 mg |
| 4 | 300 mg | 300 mg | 600 mg |
| 5 | 300 mg | 300 mg | 600 mg |
| 6 | 300 mg | 300 mg | 600 mg |
| 7 | 600 mg | 600 mg | 1200 mg |
| 8 | 600 mg | 600 mg | 1200 mg |
| 9 | 600 mg | 600 mg | 1200 mg |
| 10 | 600 mg | 600 mg | 1200 mg |
| 11 | 600 mg | 600 mg | 1200 mg |
| 12 | 600 mg | 600 mg | 1200 mg |
| 13 | 600 mg | 600 mg | 1200 mg |
| 14 | 750 mg | 750 mg | 1500 mg |
| 15 | 750 mg | 750 mg | 1500 mg |
| 16 | 1000 mg | 1000 mg | 2000 mg |

According to the XELOX regimen, capecitabine is given at 7-day intervals for 14 days. However, we continued treatment with a daily half dose to maintain tolerance during the interval period. After the successful desensitization, the patient continued to take the drug for 84 days, as prescribed by his oncologist. No hypersensitivity or toxic reactions occurred during the course of treatment. A control positron emission tomography/computed tomography scan performed after discontinuation of treatment revealed no sign of disease.

We have presented the first case of maculopapular eruptions caused by capecitabine and have described a successful desensitization protocol for this drug. Use of capecitabine in different malignancies has increased because of the advantages of outpatient treatment, and has led to increased treatment adherence, reduced treatment costs, and higher quality of life [1].

To determine which drug caused the maculopapular reaction, oxaliplatin and capecitabine were both evaluated using currently suggested methods. Although the patch tests with both drugs were negative, the provocation tests confirmed capecitabine as the culprit drug. As the alternatives are not sufficiently effective, a desensitization procedure with capecitabine was performed. The literature on desensitization is less extensive for delayed-type reactions than for immediate reactions, and it is therefore important to establish a protocol according to the drug and the patient's previous reactions [5]. In patients with exanthems, slow protocols with gradually increasing doses, which can last from hours [6] to several weeks [7], have been reported. Some patients who initially failed a rush protocol were successfully desensitized with a modified slower protocol [8]. The first clinical symptoms of a potential renewed hypersensitivity reaction may not occur for 2 to 3 days. For this reason, long protocols with repetitive, slowly increasing doses have been recommended for patients with delayed-type reactions [5]. We therefore designed a capecitabine desensitization protocol spanning 16 days for our patient.

To our knowledge, this is the first desensitization protocol described for capecitabine. Although it took 16 days to complete, the approach was safe and successful. A similar desensitization approach could be safely attempted for delayed-type hypersensitivity reactions to other oral chemotherapy agents.

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The authors declare that they have no conflicts of interest.

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## Anaphylaxis Due to Oat Ingestion

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**Key words:** Anaphylaxis. Oat. IgE-mediated hypersensitivity. SDS-PAGE immunoblotting. 48 kDa protein.

**Palabras clave:** Anafilaxia. Avena. Hipersensibilidad IgE mediada. SDS-PAGE immunoblotting. Proteína de 48 kDa.

Common oat (*Avena sativa*) is a cereal grain widely used due to its high nutritional value. It belongs to the *Poaceae* family, together with wheat, rice, barley, rye, and maize. Most patients with celiac disease can tolerate moderate amounts of pure oats, but they are advised to undergo both initial and long-term assessments by a health professional [1].

The allergic response to the inhalation of proteins from oat and cereal flour and dust (generally know as bakers' asthma and rhinitis) has been known since ancient times. Nowadays, this response is well characterized and constitutes one of the most important occupational allergies in many countries [2,3]. Contact allergy and urticaria by cutaneous sensitization due to the use of emollients/moisturizers containing oat extracts have been also described [4,5]. Patients with bakers' asthma, urticaria, and contact allergy due to oat or wheat protein usually tolerate the ingestion of oat and wheat. In the case of wheat allergy in adults, the allergen most frequently related to anaphylaxis is ω-5 gliadin [6]. However, no allergens have been described in anaphylaxis due to oat ingestion only.

We report a case of an anaphylactic reaction following oat consumption by an adult probably caused by a 48-kDa serpin protein; no allergy symptoms were observed with the ingestion of any other cereals.

The patient was a 70-year-old man with a history of essential hypertension and rheumatoid arthritis who was evaluated in our allergy unit following an acute episode of palmoplantar pruritus, generalized exanthema, nausea, vomiting, dysphonia, thoracic and laryngeal oppression, and hypotension 10 minutes after the ingestion of oat milk (200 mL). He was evaluated within an hour of the onset of symptoms. In the physical examination, the patient was conscious and oriented; he had a generalized pruritic rash, a blood pressure of 100/70 mm Hg, and a heart rate of 120 beats per minute. He was treated initially with epinephrine (1 mg/mL), dexchlorpheniramine (5 mg), and hydrocortisone (100 mg). Due to the persistence of symptoms, a new dose of epinephrine was required 20 minutes later. The patient

was taken to the emergency room and stayed there until he recovered (12 hours). He had previously tolerated oat meals and no cofactors were identified in the anaphylactic episode.

Blood cell count, biochemistry, and coagulation values were in normal ranges. Skin prick tests (SPTs) performed with the most common aeroallergens in our area and common food allergen extracts (milk, egg white and yolk, shellfish, fish, apple, nuts, tomato, garlic, and *Anisakis simplex*) were all negative.

Prick by prick tests with oat milk (8 mm) and oat seed (10 mm) were both positive. Oat seed and oat milk extracts were prepared by homogenization in phosphate buffer saline, dialyzation, and lyophilization. SPTs with oat seed extract (9 mm) and oat milk extract (8 mm) were positive; the same tests were negative in control individuals. SPTs with wheat, rice, barley, rye, and maize flour were negative.

Specific IgE determination (ImmunoCAP) to oat, barley, rye, and grass pollen were all negative (<0.35 kU/L). Serum specific IgE levels against oat seed and oat milk extracts were measured by means of the enzyme allergosorbent test (EAST method) following the manufacturer´s instructions (specific IgE EIA kit HYTEC, HYCOR BioMedical Ltd). The values obtained were 0.4 kU/L and less than 0.35 kU/L, respectively. An allergen microarray immunoassay with 112 allergens (including rTri a 14, rTri a 19, and nTri a aA_TI) (ImmunoCAP ISAC, Phadia, Thermo Fisher Scientific) was performed, with negative results in all cases.

The protein profiles of oat seed and oat milk extracts were analyzed by SDS-PAGE immunoblotting, as described by Laemmli [7]. The results showed proteins in the range of 90
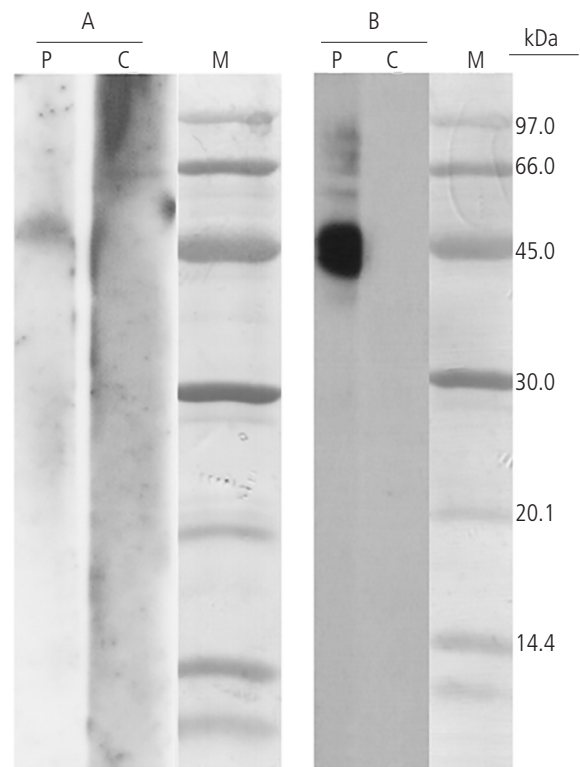


**Figure.** SDS-PAGE immunoblotting results in nonreducing conditions (without 2-mercaptoethanol). A, Oat milk extract. B, Oat seed extract. Lane P, patient serum; Lane C, control serum; Lane M, molecular mass marker.

to 14 kDa. SDS-PAGE IgE immunoblotting was carried out in nonreducing conditions and revealed a 48-kDa binding band in both the oat seed and oat milk extracts (Figure). The band was not detected when the assay was performed in reducing conditions (with 2-mercaptoethanol) (results not shown), suggesting the presence of disulphide bridges maintaining the 3-dimensional structure of this protein.

The 48-kDa-IgE-binding band from oat seed extract was extracted from the gel, digested with trypsin, and the protein was identified by mass spectrometry (MS) using LC-ESI-IT (LC-MS/MS), as previously described [8]. Sequence analysis of several internal peptides was carried out using the following sequences: DGSSVSGEEAEGLHANAEQVVQVVLADASAAGGPR, DGVFVDASLPLQSFPR, and LTTPATDVSLPLANQTR. Research conducted with protein databases identified the IgE-binding band detected as a serpin, as the protein peptide sequence yielded a strong match with serpins from related species, such as *Triticum aestivum, Triticum urartu, Oryza sativa*, and many others.

We have presented a case of anaphylaxis due to oat milk ingestion in an adult, with demonstration of an IgE-mediated mechanism and absence of cross-sensitization to other cereal antigens. The positive skin test results, as well as the positive serum specific IgE values against oat seed, demonstrated the involvement of an IgE-mediated mechanism. The results of the proteomic study pointed to a 48-kDa serpin protein from oat seed as the probable cause of the anaphylactic reaction after oat milk ingestion. The low concentration of this protein in the oat seed extract could be the cause of the low specific IgE value detected against this extract using the EAST method, and the negative result obtained in ImmunoCAP.

In one case of anaphylaxis due to oat ingestion in a child previously described by Inuo et al [9], positive results were detected against oat and wheat seed extracts in prick tests, serum specific IgE, and IgE immunoblotting. In our case, skin tests and serum specific IgE to other cereals were negative. None of the molecular masses of the IgE binding bands detected in the case described by Inuo et al were similar to the one detected by us. Furthermore, the episode of anaphylaxis occurred the first time the child consumed oat. The above facts lead us to think that the primary sensitization could be due to a cereal other than oat, unlike in our case, in which sensitization was exclusive to oat.

To our knowledge, this is the first report of anaphylaxis after the ingestion of oat with no concomitant allergy to other cereals. The results suggest that a 48-kDa serpin protein was the probable cause of this episode.

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## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Previous Presentation

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## Filaggrin Polymorphism Pro478Ser Is Associated With the Severity of Atopic Dermatitis and Colonization by *Staphylococcal aureus*

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Loss-of-function mutations in the filaggrin (*FLG*) gene are associated with increased severity of atopic dermatitis (AD) [1]. Common variants, such as p.Arg501Ter and 2282del4 may be present in up to 50% of Northern European AD patients and absent in Southern European patients [2]. rs11584340 (p.Pro478Ser) is a single-nucleotide polymorphism (SNP) of FLG that is located at codon 478. It is associated with skin barrier disruption, since the 478 serine residue may hinder the action of protease cleavage, thus affecting the rate of aggregation between FLG and keratin filaments [3,4]. Despite having a minor allele frequency of 0.34 worldwide [5], the polymorphism was found to be associated with an increased risk of AD (odds ratio, 1.87) [6,7]. Previous studies have reported that patients with null mutations in FLG have increased transepidermal water loss and increased skin pH, both of which facilitate bacterial growth [8]. However, it remains unknown how p.Pro478Ser affects predisposition to skin colonization by *Staphylococcus aureus* in AD patients.

We performed a cross-sectional analysis to evaluate the association between disease severity/colonization of the skin by *S aureus* and the polymorphism p.Pro478Ser and the null mutations in *FLG* (p.Arg501Terc and 2282del4).

Patients older than 12 years and diagnosed with AD according to the criteria of Hannifin and Rajka provided their written informed consent to participate in the study. In the case of minors, consent was given by the parents, caretakers, or guardians. The Ethics Committee of Porto University, Portugal approved the study. Participants with severe skin disease other than AD, secondary infection (bacteria, fungi, or viruses), or

any major systemic disease were excluded. Sample size was calculated based on minimal clinically important differences in the SCORing Atopic Dermatitis (SCORAD) score [9], and post hoc statistical power was set at 95.6% (P=.05) based on the prevalence of FLG mutations in a previous study of Southern European AD patients [2]. We analyzed data from 73 patients (mean age, 30 [13] years; 61% female; 77% atopic) with AD for a mean (SD) of 16 years. Severity was classified based on the SCORAD score as mild ($\leq$15), moderate (16-40), and severe ($\geq$41). Genomic DNA was extracted from peripheral blood samples and analyzed using PCR and direct DNA sequencing for the presence of the 2 null mutations in *FLG* and the p.Pro478Ser polymorphism. The microbiological profile was assessed in the right and left elbow creases, left and right popliteal creases, and neck region (area, 25 cm$^2$). The number of colony forming units (CFU)/cm$^2$ of total staphylococci and *S aureus* was determined (Baird-Parker Agar [Lab M] for total staphylococci and Mannitol Salt Agar [Lab M] for *S aureus*). The serum biomarkers assessed were total IgE, eosinophil cationic protein, and specific IgE to a mixture of inhalant allergens (Phadiatop), *S aureus* enterotoxins (A, B, C, and TSST), and *Malassezia* species (ImmunoCap). The Mann-Whitney test or Fisher exact test was used as appropriate (IBM SPSS Statistics for Windows [Version 20.0], IBM Corp).

FLG mutations were present in 15% of patients (9 with p.Arg501Ter and 2 with c.2282del4) and p.Pro478Ser in 38% (3 homozygotes, 25 heterozygotes). p.Pro478Ser was in linkage disequilibrium with the null mutations, and 3 patients with the p.Arg501Ter mutation also had p.Pro478Ser. The presence of p.Pro478Ser was associated with more severe disease, as reflected by the higher SCORAD score and severity class as well as by increased use of oral corticosteroids (Table). Furthermore, significantly more extensive colonization of *S aureus* on 3 of the 5 sampled regions and a higher value of IgE to *S aureus* enterotoxin A were observed. Homozygosity for p.Pro478Ser was not an additional risk factor in this particular group of patients. There were no differences between patients with and without the *FLG* null mutations in terms of AD severity, inflammatory allergic markers, and colonization by *S aureus*.

The novel finding of this study is that, in contrast with the 2 *FLG* null mutations, p.Pro478Ser was significantly associated with more severe disease and greater skin colonization with *S aureus* in AD patients. The 478 serine residue can increase skin permeability, leading to greater skin penetration by bacteria and conferring susceptibility to AD [4]. In addition, the presence of an unrecognized functional mutation at or adjacent to *FLG*, which is in linkage disequilibrium with p.Pro478Ser, could increase the risk for AD [10]. Therefore, our findings indicate that this SNP may have clinically relevant implications with respect to increased bacterial colonization of skin and more severe disease in AD patients.

The limitations of this study are as follows. First, the absence of healthy controls restricts us to speculation on the role of this SNP in patients with AD. Nevertheless, our objective was to study the association between this SNP and bacterial load in patients and not the role of the SNP as a risk factor for AD, in which case it would have been mandatory to include healthy controls. Second, the prevalence of *FLG*

mutations in the Portuguese population as a whole and in AD patients in Portugal is unknown. However, the sample size calculations showed that 42 patients were needed to detect a significant difference in the SCORAD score, and we were able to include more patients to overcome the level of uncertainty regarding the prevalence of genetic mutations.

Importantly, this is the first study to show an association between the presence of p.Pro478Ser and severity of AD and bacterial load in European patients with long-term AD. Only 3 previous studies have investigated this SNP, although these were in Asian patients, suggesting that it confers susceptibility to AD, particularly in patients with high IgE levels [3,6,7]. The low prevalence of FLG null mutations in our study is consistent with the wide variation in this gene mutation across the globe and the lower prevalence in Southern European countries. The lack of an association with clinical, microbiological, and allergic parameters reinforces the fact that genetic markers other than FLG mutations should be studied.

In conclusion, genetic factors can affect the severity of AD and skin microbiota. Our study shows that the presence of p.Pro478Ser may be related to both increased disease severity and bacterial colonization in patients with long-term AD.

### Funding

**Table.** Characteristics of Patients With Atopic Dermatitis According to Filaggrin Genotype[a]

| | FLG Null Mutations Mp.Arg501Ter or C.2282del4 | | | FLG Polymorphism Pro478Ser | | |
|---|---|---|---|---|---|---|
| | Yes, n=11 | No, n=62 | P Value | Yes, n=28 | No, n=45 | P Value |
| Age, y | 32 (6.1) | 29.6 (1,5) | .91[b] | 34.1 (2.7) | 27.3 (1.8) | .03[b,d] |
| Female sex, No. (%) | 7 (63.6) | 38 (61.5) | .22[c] | 16 (57.1) | 28 (62.2) | .42[c] |
| Disease duration, y | 15.9 (10.5) | 16.3 (10.4) | .23[b] | 18.4 (2.3) | 14.8(1.3) | .32[b] |
| SCORAD (0-103) | 50.2 (30.9) | 41.3 (22.6) | .72[b] | 51.8 (4.2) | 36.0(3.4) | <.01[b,d] |
| SCORAD severity, No. (%) | | | | | | |
|   Mild | 2 (18.2) | 5 (8.1) | .28[c] | 2 (7.1) | 5 (11.1) | .40[c] |
|   Moderate | 3 (27.3) | 26 (41.9) | .81[c] | 6 (21.4) | 23 (51.1) | .02[c,d] |
|   Severe | 6 (54.5) | 31 (50.0) | .52[c] | 20 (71.4) | 17 (37.8) | .01[c] |
| Oral corticosteroids, No. (%) | 3 (27.3) | 30 (48.4) | .22[c] | 17 (60.7) | 16 (35.6) | .03[c,d] |
| Atopic, No. (%) | 6 (54.5) | 50 (79) | .53[c] | 22 (78.6) | 34 (75.6) | .52[c] |
| Asthmatic, No. (%) | 4 (36.4) | 36 (58.1) | .64[c] | 14 (50.0) | 26 (57.8) | .31[c] |
| Median (IQR) total IgE, IU/mL, | 2185 (71.4-5308) | 4183 (97.3-3607.8) | .08[b] | 6520 (113.6-7935.0) | 2240 (88.6-1151.5) | .08[b] |
| Median Phadiatop, $kU_A/L$ median ($P_{25-75}$) | 248.6 (4.5-565.9) | 529.9 (0.54-441.0) | .12[b] | 763 (9.6-1115.9) | 315 (0.4-283.5) | .13[b] |
| ECP, µg/L | 20.7 (14.9) | 35.2 (29.1) | .56[b] | 37.2 (34.2) | 30.5 (21.1) | .52[b] |
| Specific IgE, $kU_A/L$ | | | | | | |
|   Enterotoxin A | 0.37 (0.2) | 2.4 (1.3) | .79[b] | 4.5 (13.9) | 0.5 (0.9) | .05[b,d] |
|   Enterotoxin B | 0.6 (0.3) | 1.5 (0.5) | .42[b] | 2.4 (5.1) | 0.6 (1.3) | .23[b] |
|   Enterotoxin C | 1.3 (0.5) | 2.2 (0.5) | .38[b] | 2.7 (3.5) | 1.6 (3.1) | .06[b] |
|   Enterotoxin TSST | 0.5 (0.2) | 1.4 (0.6) | .52[b] | 2.4 (6.7) | 0.4 (0.8) | .08[b] |
|   Malassezia species | 6.2 (5.8) | 4.2 (1.1) | .78[b] | 7.2 (13.4) | 3.3 (8.7) | .23[b] |
| Staphylococcus aureus, $CFU/cm^2$ | | | | | | |
|   Right arm | 9471.1 | 78 152.7 | .48[b] | 178 083.3 | 8002.3 | .01[b,d] |
|   Left arm | 158 909.9 | 70 271.9 | .58[b] | 142 859.2 | 48 310.3 | .92[b] |
|   Right leg | 23 454.4 | 39 728.2 | .91[b] | 89 778.9 | 8 386.7 | .04[b,d] |
|   Left leg | 162 754.4 | 359 865.8 | .96[b] | 759 552.7 | 95 528.5 | .02[b,d] |
|   Neck | 8 994.9 | 30 732.6 | .74[b] | 48 538.3 | 16 244.8 | .80[b] |

Abbreviation: ECP, eosinophil cationic protein; SCORAD, SCORing Atopic Dermatitis.
[a]Results are presented as mean (SD) unless stated otherwise.
[b]Mann-Whitney test.
[c]Fisher exact test.
[d]Statistically significant.

*Conflicts of Interest*

The authors declare that they have no conflicts of interest.

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