Anti-Amoxicillin Immunoglobulin E, Histamine-2 Receptor Antagonist Therapy and Mast Cell Activation Syndrome Are Risk Factors for Amoxicillin Anaphylaxis

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Key Words

Amoxicillin · Anaphylaxis · Immunoglobulin E ·
Gastroprotective drugs · Mast cell activation syndrome

Abstract

Background: β -Lactam antibiotics (mainly amoxicillin, AX) are the drugs that most frequently induce systemic drug allergy reactions. **Objective:** We attempted to identify the risk factors associated with systemic reactions to AX. **Methods:** All patients who were referred to our department for suspected hypersensitivity reactions to AX over a 6-month period were evaluated for anti-AX immunoglobulin E (IgE) levels and skin-test positivity for β -lactams. Age, sex, concomitant diseases, therapies, total IgE, serum tryptase levels and signs and symptoms suggesting mast cell activation syndrome (MCAS) were analyzed in relation to the severity of the reaction in accordance with the Mueller classification. **Results:** Sixty-seven patients were selected: 39 with mild reactions such as cutaneous or gastrointestinal symptoms (grades I and II) and 28 with severe systemic reactions (grades III and IV). Anti-AX IgE levels and total IgE were significantly higher in severe reactions than in mild ones ($p < 0.00005$, $p =$

0.0037). Treatment with histamine-2 receptor antagonists (anti-H2) was significantly related to severe reactions ($p = 0.007$). No significant correlations were found between the severity of the reactions and dyslipidemia or levels of angiotensin-converting enzyme and tryptase. **Conclusion:** Anti-AX IgE levels were the most significant immunological parameter distinguishing patients who presented with severe reactions to AX and those with mild reactions. Higher values of total IgE, the use of gastroprotective drugs and signs and symptoms suggesting an MCAS significantly increased the odds ratio of having a severe reaction. The risk of serious adverse reactions to AX increased in older patients and in males, but this trend was not significant.

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Introduction

Anaphylaxis is a life-threatening reaction. Several epidemiological studies indicate that this phenomenon has been increasing for at least 10 years. In particular, studies conducted in Australia, the UK and USA showed an increase in the anaphylaxis rate from 1990 to 2006 [1–4]. A

recent US study described a similar trend, and also showed that drug allergy is the leading cause of anaphylaxis-related deaths [5]. The data confirmed the results of an Australian retrospective study [6], which showed that the anaphylaxis mortality rate remained stable from 1997 to 2005 (0.64 deaths per million population per year), with the exception of drug-induced anaphylaxis deaths, which have increased. Several studies concerning the causes of fatal or almost-fatal anaphylaxis in the USA, Australia, Asia and Europe agree in confirming that in the early years of life, food is the most common trigger of anaphylaxis. In contrast, drug allergy is the most important cause in adults, especially in the age group of 55–84 years, with a predominance in males. With regard to the most commonly involved drugs, Lauritano et al. [7] recently studied adult patients admitted to a sub-intensive care unit for drug anaphylaxis, and found that antibiotics were implicated in 37.1% of the cases, with a predominance of penicillins (56.4%). Similarly, Moro et al. [8] found that antibiotics, especially amoxicillin (AX)-clavulanic acid and AX, were the principal causes of drug-induced anaphylaxis in 25% of the cases of older patients. Selective allergy to AX or ampicillin (AM) is relatively common in southern Europe but is infrequent in the USA; the reason for this difference is not known [9]. An important issue in anaphylaxis is the set of risk factors that can condition the severity of the allergic reaction. In children, allergic asthma is the most important factor [10]. In contrast, in elderly adults, chronic concomitant diseases and concurrent medications may predispose to fatal reactions [11]. However, the roles of baseline tryptase and the newly described mast cell activation syndrome (MCAS) have never been evaluated [12]. The aim of our study was to identify the clinical aspects predisposing adult subjects to severe systemic reactions to AX in a study population of patients who were evaluated after a recent episode of AX-induced allergic reaction.

Methods

Patients

All of the subjects (18–77 years old) who were admitted to our unit from January to June 2012 for a history of AX-induced systemic reaction of any severity in the previous 6 months were enrolled in the study for subsequent analysis. On the basis of a standard questionnaire, we collected data that included the type of reaction, the severity, any delay with respect to drug intake and the number of episodes. Atopy was considered positive on the basis of a family or personal history of allergic diseases confirmed by skin test positivity to any food or inhalant allergen [13]. AX-induced systemic reactions were classified as immediate (≤ 1 h) and nonimmediate (>1 h) following European Network of Drug Allergy rec-

ommendations [14, 15]. The severity of symptoms was classified in accordance with the Mueller [16] classification. For convenience, we considered morbilliform rash and urticaria as grade I reactions, even if they were evaluated separately for the statistical analysis. All of the patients were enrolled after signing an informed consent form for data to be collected. The patients were tested for serum tryptase and specific immunoglobulin E (IgE) towards β -lactams. If these were negative, the patients were subjected to skin tests, i.e. skin prick tests (SPTs) and intradermal tests (IDTs) with penicillin reagents, according to European Network of Drug Allergy protocols [14, 15]. In the case of severe anaphylaxis, *in vitro* tests were considered the only diagnostic choice in order to avoid the risk of severe reactions by skin testing as recently shown [17]. For all the selected patients, statistical analysis was used to evaluate the association between the risk of severe AX-induced reactions and atopy, age, gender, anti- β -lactam IgE levels, dyslipidemia, atherosclerosis, the use of histamine-2 receptor antagonists (anti-H₂), proton-pump inhibitors (PPIs) and serum angiotensin-converting enzyme (ACE) therapy, levels of serum ACE and basal serum tryptase and the signs and symptoms of MCAS. Tryptase was also measured when a symptomatic event occurred that was not related to a drug reaction.

In vitro Tests

Specific IgE levels against penicilloyl V, G, ampicilloyl, amoxicilloyl and cefaclor and total IgE levels were determined in each patient using the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden) in accordance with the manufacturer's instructions. Specific IgE values were considered positive when a value ≥ 0.10 kUA/l was obtained. A positive cut-off value of total IgE was considered to be >100 kUA/l. Tryptase was measured with an immunofluorimetric assay (ImmunoCAP). One measurement of tryptase levels was performed for all of the patients during an asymptomatic period, at least 6 weeks after the last anaphylactic/systemic allergic event. This test was repeated only in some selected cases during a symptomatic phase (urticaria). The cut-off value of tryptase was considered to be ≥ 5 ng/ml, according to the in-house reference obtained from a sample of hundreds of healthy volunteer subjects.

In vivo Tests

SPTs and IDTs were conducted with the major determinant penicilloyl-polylysine (Diater Laboratorios, Madrid, Spain) and the minor determinant mixture consisting of sodium-benzylpenicillin, benzylpenicilloic acid and sodium-benzylpenicilloate (Diater Laboratorios), AX and AM. In the case of a negative SPT with undiluted reagents after 20 min, IDTs were performed with penicilloyl-polylysine and minor determinant mixture with dilutions of 1:100, 1:10 and also undiluted. As penicillin G is not routinely available in the pharmacy of our hospital, we performed the test with penicillin G using concentrations of 1,000 IU/ml and 10,000 IU/ml only in some patients. The concentrations for both AM and AX were 1 mg/ml and, if negative, 20 mg/ml. IDTs were performed using 0.02 ml of solution prepared daily. Histamine 10 mg/ml (ALK-Abello, Hørsholm, Denmark) and isotonic sodium chloride were used as controls. In the case of nonimmediate hypersensitivity reactions, epicutaneous testing (patch tests) was also performed with penicillin G, AM and AX (5% in petrolatum). Patch tests were measured 15 min after the removal of the strips after 48 h of occlusion time [18], in accordance with the European Environmental and Contact Dermatitis Research Group patch-test classification.

Statistical Analysis

All of the collected variables were subjected to descriptive analysis. Continuous variables were described by mean and standard deviation or by median and range, based on their distribution (checked by visual inspection of the histogram and by the Shapiro-Wilk test), while categorical variables were described by relative and absolute frequency tables. Cross-tabulations between categorical variables were analyzed with the Fisher's exact test. The differences in continuous variables with respect to reaction severity were analyzed by means of the two-sided Mann-Whitney U test. The continuous variables of interest as well as possible confounders like age and sex were then fitted as independent regressors in a set of logistic models, using the Wald's test to verify the significance of each regressor and the likelihood ratio test to check the significance of the model as a whole. In these logistic regressions, the reaction severity was taken into account as a dependent binary variable. To obtain a reliable cut-off of continuous variables, receiver operating characteristic (ROC) curve analysis was carried out, and the Youden method was used to choose for the optimal cut points. Statistical significance was assumed for $p < 0.05$; all calculations were carried out using the Stata/SE 13.1 statistical package.

Results

Patients

A total of 67 patients (12 males and 55 females, mean age 44.9 ± 16.0 years) with a clear-cut history of systemic reactions to AX were included in this study. A total of 32/67 patients (48%; 5 males and 27 females) had grade I reactions, 7/67 patients (10%) had grade II reactions, 10/67 patients (15%; 2 males and 8 females) had grade III reactions and 18/67 patients (27%; 5 males and 13 females) had grade IV reactions. For the statistical analysis, given that our main objective was the identification of the risk factors predisposing to anaphylactic reactions to AX, the patients were further classified into 2 groups. Group A consisted of 39 patients (58%; mean age: 41.6 ± 15.7 years) with grade I and II reactions, considered as the control group. Group B, representing our series of patients, consisted of 28 patients (42%; mean age: 49.5 ± 15.7 years) with grade III and IV reactions. Twelve out of 31 patients (39%) with grade I reactions had suffered from a morbilliform rash and 19 (61%) had suffered from urticaria. The characteristics of the patients are summarized in table 1. All the considered clinical parameters were compared between groups A and B.

Association between the Different Clinical and Biochemical Aspects and the Severity of the Reactions

Atopy. A total of 22/67 patients (32.8%) reported a positive family history of allergic disease and 19/67 (28.35%) had a personal history of allergic disease. A fam-

Fig. 1. Association between anti-AX-specific IgE values and type of reaction.

Table 1. Patients' characteristic

	n	%
Total number of patients	67	
Mean age, years (range)	44.9 (18–77)	
Males/females	12/55	
Family history of allergic disease	22	32.8
Personal history of allergic disease	19	28.35
Severity of reaction (males/females)		
Grade I	32 (5/27)	47.77
Grade II	7 (0/7)	10.44
Grade III	10 (2/8)	14.92
Grade IV	18 (5/13)	26.87
Concomitant diseases		
Asthma	9	13.4

ily history of atopy increased the odds of severe allergic reactions to AX by 260% (Wald's test: $p = 0.020$). Personal atopy was not associated with a severe reaction (Wald's test: $p = 0.667$).

Age and Gender. Each additional year of age increased the risk of severe reactions by 3.3% with a low significant effect (Wald's test: $p = 0.05$). No differences in odds of severe reactions were found due to gender (Wald's test: $p = 0.207$).

Specific Anti-AX IgE Serum Levels. Levels were positive in 27/67 patients (40.3%), i.e. 6/39 (15%) in group A (5 had a grade I reaction and 1 had grade II) and 21/28 (75%) in group B (5 had a grade III reaction and 16 had grade IV). The risk of severe anaphylactic reactions increased significantly with rising anti-AX IgE levels (Mann-Whitney U test: $p < 0.00005$; fig. 1). Logistic regression analysis

Fig. 2. Association between anti-penicilloyl-G-specific IgE values and type of reaction.

was performed to study the impact of anti-AX-specific IgE levels on the severity of the reaction, and revealed that every single unitary increase of the value of the anti-AX IgE levels increased the odds (Wald's test: $p = 0.020$) of severe reactions by 155%. A significant correlation was also detected between anti-AX IgE levels and the severity of the reaction based on multivariate analysis after adjusting for age and sex (Wald's test: $p = 0.012$); in this model, the odds of severe reactions increased by 166% for any unitary increase in anti-AX IgE values.

Specific Anti-Penicilloyl G and V IgE Serum Levels. Specific anti-penicilloyl G IgE levels were positive in 15/67 patients (3%), i.e. 2/39 (5.12%) in group A (2 had a grade I reaction) and 13/28 (46.42%) in group B (4 had a grade III reaction and 10 had grade IV). The risk of severe anaphylactic reactions increased significantly with rising anti-penicilloyl G IgE levels (Mann-Whitney U test: $p = 0.0007$; fig. 2). Specific anti-penicilloyl V IgE levels were positive in 20/67 patients (29.85%), i.e. 4/39 (10.25%) in group A (3 had a grade I reaction and 1 had grade II) and 16/28 (57.14%) in group B (3 had a grade III reaction and 13 had grade IV). The risk of severe anaphylactic reactions increased significantly with rising anti-penicilloyl V IgE levels (Mann-Whitney U test: $p < 0.00005$; fig. 3).

Performance Characteristics for Different Cut-Off Points of Specific Anti-AX and Anti-Penicilloyl G and V IgE Values. We tried to calculate the positive predictive value (PPV) and negative predictive value (NPV) together with sensitivity and specificity for serum IgE to AX and

Fig. 3. Association between anti-penicilloyl-V-specific IgE values and type of reaction.

Table 2. Diagnostic characteristics of various possible cut-off points for AX-specific IgE, penicilloyl G-specific IgE and penicilloyl V-specific IgE

Cut-off points, kUA/l	PPV, %	NPV, %	Sensitivity, %	Specificity, %	Accuracy, %
ImmunoCap for AX					
0.12	78.6	84.6	78.6	84.6	83.3
0.44	78.9	72.9	53.6	89.7	75.8
2	70.0	63.2	25.0	92.3	65.2
3.46	83.3	62.3	17.9	97.4	65.2
ImmunoCap for penicilloyl G					
0.20	80.0	69.2	42.9	92.3	72.7
0.57	83.3	67.3	35.7	94.9	71.2
1.66	75.0	62.7	21.4	94.9	65.2
3.79	50.0	58.7	7.10	94.9	59.1
ImmunoCap for penicilloyl V					
0.14	76.5	70.0	46.4	89.7	76.9
0.24	81.3	70.6	46.4	92.3	75.4
0.39	84.6	68.5	39.3	94.9	73.9
2.03	71.4	61.7	17.9	94.9	64.6

penicilloyl G and V for different cut-off points (table 2). If we decided to use the lowest cut-off values for specific IgE to AX, penicilloyl G and V all together, we would find a cut-off value ≥ 0.20 kUA/l, a PPV of 92.9% (95% CI 66.1–99.8), an NPV of 71.7% (95% CI 57.7–83.2), a sensitivity of 46.4% (95% CI 27.5–66.1) and a specificity of 97.4% (95% CI 86.5–99.9), with a greater possibility of identification an AX-induced allergic reaction.

Serum Total IgE Levels. There was a significant correlation between total IgE levels and reaction severity ($p = 0.0037$; fig. 4). In particular, there was a 77% probability (for 2 subjects who were randomly chosen, 1 from group A and 1 from group B) that the subject from the group with the severe reactions had higher total IgE values; however, any unitary increment of the total IgE increased the odds of having a severe response by 0.49%.

Skin Tests for Penicillin Determinants. We performed skin tests on a group of 32 patients (26 from group A and 6 from group B) with negative specific IgE for all of the β -lactamic determinants. In group A, there were 8/26 (31%) positives, i.e. 8/8 with positive IDTs and 2/8 with positive patch-test results. In group B, we detected 3/6 (50%) with positivity (IDT 3/3; Fisher's exact test: $p = 0.390$). IDT positivity was immediate (within 20 min) in 4/8 patients (50%) in group A and 3/3 patients (100%) in group B (Fisher's exact test: $p = 0.236$).

Impact of Anti-H2 Therapy on Anti-AX IgE Levels and Reaction Severity. A total of 17 patients (6 from group A and 11 from group B) had received anti-H2 therapy during AX treatment. This therapy significantly increased the risk of severe reactions (Fisher's exact test: $p = 0.007$), even after adjusting for age (Wald's test: $p = 0.032$). Adjusting for anti-H2 therapy, the odds of having a severe reaction to AX increased by 107% for each unitary increase in anti-AX IgE (Wald's test: $p = 0.037$). We found that patients who had received anti-H2 therapy presented higher levels of anti-AX IgE levels (Mann-Whitney U test: $p = 0.0261$).

PPI Therapy. This therapy was much more frequent in patients with severe allergic reactions (Fisher's exact test: $p = 0.011$). Adjusting for age, this correlation was not actually significant (Wald's test: $p = 0.053$); it nevertheless displayed a trend to increase the risk of severe reaction.

Impact of MCAS Signs and Symptoms on the Risk for Severe AX-Induced Reactions. The unitary increase of the number of signs or symptoms (table 3) increased the odds of having a severe reaction by 34% (Wald's test: $p = 0.021$); after adjusting for atopy, the odds increased by 46% (Wald's test: $p = 0.008$). The basal levels of tryptase were measured in 56/67 patients, i.e. 14/56 (25%) had values between 5 and 12 ng/ml, and in another 10/67 patients (14.92%), the tryptase levels measured during episodes of urticaria that were not drug-induced increased up to 14.6 ng/ml, representing >20% of the basal levels plus absolute 2 ng/ml. Basal serum tryptase (Mann-Whitney U test: $p = 0.3674$) and elevated basal serum tryptase (i.e. ≥ 5 ng/ml) were not associated with the se-

Fig. 4. Association between total IgE values on logarithmic scale and type of reaction.

Table 3. Signs and symptoms suggestive of MCAS

Skin	Flushing Rash Itching Hives
Gastrointestinal	Nausea Vomiting Abdominal cramping Diarrhea Gastroesophageal reflux Bloating Malabsorption
Cardiovascular	Hypotension Syncope or near syncope Tachycardia
Respiratory	Wheezing
Nasal-ocular	Conjunctival injection Itching Nasal stuffiness
Neurological	Memory, concentration difficulties and headache
Decrease in frequency, severity or resolution of symptoms with antimediation therapy	Therapy with H1 and H2 histamines receptor inverse agonists, antileukotriene medications or cromolyn sodium

verity of the reaction, based on logistic regression (Wald's test: $p = 0.420$).

We could not identify any association between the severity of the AX-induced allergic reactions and dyslipidemia (Wald's test: $p = 0.491$), atherosclerosis (Fisher's ex-

act test: $p = 0.113$), serum ACE inhibitor treatment (Fisher's exact test: $p = 0.140$) and serum ACE level (Mann-Whitney U test: $p = 0.4043$).

Discussion

In this study, we identified several risk factors that condition the occurrence and severity of systemic hypersensitivity reactions to AX. Our major finding was that anti-AX IgE levels were the most significant immunological parameter for identifying the grade of severity. The levels of anti-AX IgE antibodies were significantly higher in severe reactions than in mild reactions ($p < 0.00005$). We found IgE positivity in 75% of the severe reactions because we included only patients who had presented reactions in the previous 6 months. In contrast, as expected, only 15% of the patients with mild reactions (group A) had measurable IgE levels. Interestingly, 2 of 4 patients with grade 1 reactions and positivity for anti-AX IgE had a morbilliform rash and the other 2 had urticaria.

In group B, only 6/28 patients had negative anti-AX IgE antibodies; 3 of these presented with skin-test positivity. Thus, in group B, 25/28 patients (89.28%) had signs of anti-AX IgE antibody positivity. We can conclude that almost 90% of the severe systemic reactions to AX, if tested close to the reaction, presented with positive values of anti-AX IgE antibodies in the skin tests. Thus, in clinical practice, it is very important to evaluate patients as soon as possible after an AX reaction. The values of specific IgE anti-penicilloil G and V were also statistically correlated with the trend of the severity of the reaction. According to ROC analysis, the optimal cut-off level was obtained considering specific IgE to AX and penicilloil G and V simultaneously, and was 0.20 kUA/l for each. Another relevant point is that the use of gastroprotective drugs (and in particular, anti-H2 drugs) during a course of AX therapy is a very significant risk factor for having a severe systemic reaction to AX ($p = 0.007$). Thus, in patients on anti-H2 therapy, the odds of having a systemic reaction to AX increases by 107% ($p = 0.037$). Furthermore, we observed that anti-H2 therapy plays a role in inducing increased anti-AX IgE antibody levels. In fact, patients who were treated with anti-H2 presented significantly higher levels of anti-AX IgE antibodies ($p = 0.0261$) than those not treated with anti-H2. This relationship is new for AX allergy but not for other models of allergic disease, e.g. food allergy. Untersmayr and Jensen-Jarolim [19] found that interference with the gastric-digestion capacity by antacids (which increase the gastric pH, leading to

the persistence of labile food protein during gastric transit), influenced the predisposition to develop severe allergic reactions in already-sensitized patients who were allergic to melon. Greenberger and Patterson [20] reviewed the premedication protocol recommended for iodinated contrast-media administration in high-risk patients and demonstrated that the addition of the anti-H2 cimetidine resulted in a slight increase in the repeat reaction rate. Recent guidelines recommend that the use of anti-H2 treatment be limited [21]. In addition, Ramírez et al. [22] demonstrated that in hospitalized patients, the use of PPIs was associated with a significantly increased risk of drug hypersensitivity reactions, similar to a personal history of drug allergies and a long hospitalization time. All of these findings suggest that suppression of the H2 receptors can decrease the ability to degrade allergenic molecules in the stomach and can thereby lead to a lack of degradation of resistant allergens such as AX. It is relevant to keep in mind that the opening of the β -lactam ring occurs in the stomach.

With regard to the other findings, we also observed that a personal history of atopy, gender and age, serum ACE level, dyslipidemia, atherosclerosis and therapy with ACE inhibitors did not significantly modify the risk of having a severe allergic reaction to AX. In addition, in accordance with the recent literature, anaphylaxis to drugs does not present a gender-related predisposition. Anaphylaxis is more common in elderly individuals. According to our results, male gender increases the odds of having a severe response by 127% ($p = 0.207$). However, Liew et al. [6] reported a preponderance of female subjects for drug-induced anaphylaxis and an equal sex distribution in the case of fatal anaphylaxis. A study of gender as a risk factor for allergy to penicillin identified a significant association between the female sex and allergy to penicillin [23].

In the model of AX hypersensitivity, in contrast with data on hymenoptera venom allergy, we did not find that basal levels of tryptase could predispose one to a more severe reaction. However, we did find a correlation between the criteria for the diagnosis of MCAS [24] and AX allergy. We found that the addition of every single sign or symptom of MCAS increased the odds of presenting a severe reaction by 34% ($p = 0.021$). A diagnosis of MCAS, despite the absence of a consensus, is assigned to some patients with a variable number of unexplained signs and symptoms likely related to histamine release. Patients presenting signs and symptoms that suggest MCAS have generally undergone an extensive evaluation to rule out known diseases. Although tryptase levels in-

crease during mast cell activation, it is recognized that a normal tryptase level does not rule out a clonal mast cell disease. In fact, in our patients with suspected MCAS, we did not find high basal values of tryptase. However, in 10 cases, there was a transient increase of tryptase in the course of acute urticaria (of at least 1 month), thus confirming the current MCAS diagnostic criteria. A limitation of our study could be that the patients were selected only on the basis of their clinical history which is not always reliable.

In conclusion, anti-AX IgE levels, total IgE values, gastroprotective drugs (anti-H2 or PPIs) and signs and symptoms of MCAS are the main risk factors for severe allergic reactions to AX.

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