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The Importance of Amoxicillin and Amoxicillin-Clavulanate Determinants in the Diagnosis of Immediate Allergic Reactions to β-Lactams

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Key Words
Penicillin · β-Lactam · Allergy · Skin test

Abstract
Background: Immediate allergic reactions to β-lactam antibiotics are considered to be one of the most important drug hypersensitivities. A positive skin test (ST) with a combination of major and minor penicillin determinants is usually sufficient to recommend avoidance of the culprit drug, whereas a negative ST is usually followed by an oral challenge test (OCT). Recently, concern has been raised regarding the role of amoxicillin (AMX) ST in the diagnosis of AMX allergy. ***Objective:*** The aim of this study was to examine the additive value of AMX determinants in STs of patients with immediate hypersensitivity reactions to AMX or AMX-clavulanate (AMX-C). ***Methods:*** Patients with a history of immediate AMX or AMX-C allergy underwent an ST using a combination of penicilloyl-polylysine (PPL) and minor determinants as well as AMX. An ST with AMX-C was added when appropriate. ***Results:*** Thirty-one patients were evaluated. Eight patients, all of them with a history of AMX allergy, had positive reactions only to the AMX component. Two patients with AMX-C allergy had a positive ST reaction only to the AMX-C component. Moreover, only 14 patients (13 with AMX and 1 with AMX-C allergy) had a positive reaction to PPL, whereas most patients (54.8%) had positive reactions to other determinants. One patient, who was positive for AMX, developed several urticarial lesions after the test. ***Conclusions:*** Skin testing with AMX and AMX-C is mandatory in patients with immediate allergy to these drugs. Failure to perform it may result in a false-negative ST jeopardizing these patients with anaphylactic reactions during a hazardous OCT.

Introduction

Allergy to β-lactam (BL) antibiotics is considered to be a major public health issue. Although epidemiological data suggest that up to 8% of the population have a positive history of BL allergy, only a small portion of these patients have true allergy [1]. Mistakenly labeling a patient with allergy to BL has a major impact and may affect morbidity and even mortality [2]. Thus, it is important to use standardized objective measures in order to confirm this medical condition. There are two forms of BL hyper-

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sensitivity, 'immediate' and 'nonimmediate' [3]. The immediate, IgE-mediated allergy consists of severe, potentially life-threatening anaphylactic reactions accruing within 1 h after having taken the offending drug. The nonimmediate hypersensitivity reaction can appear within <24 h from BL administration and generally consists of a non-life-threatening rash [4–6].

In order to promptly diagnose IgE-mediated hypersensitivity, a skin test (ST) followed by an oral challenge test (OCT), administered under strict medical supervision, is warranted [7]. Some institutions use the basophil activation test when there is a high clinical suspicion of IgE-mediated hypersensitivity but negative ST results, but this test is not available in most centers [8]. A ST is done using several penicillin determinants, including the FDA-approved major determinant penicilloyl-polylysine (PPL) together with some minor determinants including penicillin G, and another minor determinant mixture (MDM), containing benzylpenicillin, benzylpenicilloic acid, and benzylpenicilloate, which is available in Europe. Some groups also use amoxicillin (AMX) with or without AMX-clavulanate (AMX-C) for skin testing, depending on the culprit drug [9]. Unfortunately, no consensus exists regarding the optimal ST regimen and OCT protocol. In patients suspected of having immediate, IgE-mediated hypersensitivity to BL, performing STs with all available determinants (PPL, MDM, penicillin G, and AMX) followed by OTC, when the ST is negative, is the common practice in part of the medical centers, especially in Europe [9]. Nevertheless, concern has been raised regarding the need for using both MDM and AMX as ST reagents [9, 10]. In the USA, it has been shown in a very large group of unselected patients with a history of penicillin allergy that they could safely be evaluated with a protocol using skin testing only with PPL and penicillin G followed by an oral AMX challenge if the ST was negative [10, 11].

The aim of our study was to examine the safety and additive value of AMX and AMX-C determinants when skin testing a small, highly selected group of penicillin-allergic individuals who had both a positive history of immediate hypersensitivity to AMX and/or AMX-C and a positive penicillin ST.

Methods

Patients and Study Design

This was a prospective study conducted between 2011 and 2015 in the Allergy and Clinical Immunology Unit at the Meir Medical Center, Kfar Saba, Israel. The study included all subjects referred to our outpatient clinic for evaluation of BL hypersensitivity.

Inclusion criteria were a positive history of an immediate reaction to AMX or AMX-C together with a positive ST reaction to at least one of the tested determinants, regardless of the age of the patient. An immediate reaction was defined as clinically relevant signs or symptoms: urticaria, pruritus, angioedema, gastrointestinal symptoms, dyspnea, or anaphylactic shock (at least involving two different systems) starting within 1 h after drug administration.

Exclusion criteria included nonimmediate reactions to penicillin starting longer than 1 h after drug administration, a positive history of allergy to another BL (i.e. cephalosporins), reactions starting at an unknown time after drug administration, and patients with no recollection of the hypersensitivity reaction that, for unknown reasons, was 'tagged' as allergic to penicillin. Patients with a positive history of an immediate reaction but with negative ST results to all tested determinants were also excluded from the current study. This study was approved by the local ethics committee.

Skin Tests

All subjects were thoroughly interviewed by an allergist in order to properly define the suspected hypersensitivity type. Afterwards, all patients underwent an ST with PPL (1:10 and 1:1), MDM (1:10 and 1:1), and AMX (1:10 and 1:1) (Diater, Madrid, Spain) and penicillin G 10,000 U/ml (Teva, Israel). If the culprit BL was AMX-C, patients were also tested with AMX-C 20 mg/ml (Augmentin; GlaxoSmithKline, Brentford, UK). Normal saline and histamine phosphate (Histatrol; ALK, Washington, N.Y., USA) (2.75 and 0.275 mg/ml for prick and intradermal tests, respectively) served as negative and positive controls, respectively. The ST was considered positive if the longest wheal diameter was ≥5 mm compared to the control, together with a positive flare.

A prick ST was performed initially, and if negative, an intradermal ST was done, first with the lower concentrations and, if negative, with the higher concentrations. ST results were read after 15 min by the allergist. After ST results had been read, patients were observed for 1 h, and a follow-up telephone interview was done 1 week later in an attempt to document adverse effects related to the ST.

Statistical Analysis

Data were entered into and tabulated using Excel 2007. Statistical analyses were performed using SPSS version 19 software. Data are presented as means and standard errors for continuous variables. Comparisons between groups were performed with Student's t test for continuous variables and with Fisher's exact test for categorical variables. All tests of hypotheses were considered significant when two-sided probability values were $p < 0.05$.

Results

A total of 796 patients with alleged BL hypersensitivity were evaluated. Eighty-nine patients (11.2%) had a positive history of an immediate allergy to a BL. Twenty-nine patients (3.6%) had a negative ST to all determinants tested and were excluded from the present study. The remaining 60 patients (7.5%) had a history compatible with an immediate IgE-mediated allergy combined with a pos-

Table 1. Patients' characteristics

	Overall (n = 31)	Positive ST to AMX (n = 16)	Negative ST to AMX (n = 15)	p value
Male gender	15 (48.3)	8 (50)	7 (46.6)	1
Age, years	20.1±22.8 (0.75–72)	24.6±27	15.4±17.1	0.2
Time interval[1], months	18±63.6	9.9±14.1	30.8±91.2	0.3
Type of reaction				
Urticaria/angioedema	29 (93.5)	16 (100)	13 (86.6)	0.2
Anaphylactic shock	8 (25.8)	5 (31.2)	3 (0.2)	0.6
Dyspnea	11 (35.4)	6 (37.5)	5 (33.3)	1
Vomiting/abdominal pain	8 (25.8)	5 (31.5)	3 (20)	0.6
Culprit drug				
AMX	24 (77.4)	13 (42)	11 (35.5)	1
AMX-C	7 (22.6)	3 (9.6)	4 (12.9)	

Values are n (%) or means ± standard deviations (ranges). [1] Time elapsed between the allergic reaction and the ST.

itive ST. Thirty-one patients (3.9%) had both a positive history of an immediate allergy to AMX or AMX-C and a positive ST to at least one of the determinants and were included in our study. The remaining 29 patients had a positive history of nonpenicillin BL (i.e. cephalosporin) and, hence, were excluded from the current study. ST was positive in 6 patients (19.3%) by prick, in 5 patients (16.1%) by intradermal testing with the lower concentration, and in 20 patients (64.5%) by intradermal testing with the higher concentration. Patients' characteristics are presented in table 1. The culprit drug was AMX in 24 subjects (77.4%). Urticaria, with or without angioedema, was the most frequent clinical sign, appearing in 29 patients (93.5%) within 1 h from taking the offending drug. The age distribution of the patients was wide, ranging from 9 months up to 72 years.

Sixteen patients (51.6%) had a positive ST to the AMX component. Eight patients (25.8%), all of whom had a history of AMX allergy, reacted only to the AMX component (fig. 1). Moreover, only 14 patients (45.1%), 13 with AMX and 1 with AMX-C allergy, had a positive reaction to PPL, whereas the majority of the patients (54.9%) had a positive reaction to one or more determinants other than PPL (fig. 1). Only 2 patients (6.5%), 1 with AMX and 1 with AMX-C allergy, had a positive reaction to penicillin G. Two out of 7 patients with AMX-C allergy had a positive ST reaction only to AMX-C, 3 patients had a positive reaction to AMX and AMX-C, 1 patient had positive reactions to AMX-C and penicillin G, and 1 patient had a positive reaction only to PPL.

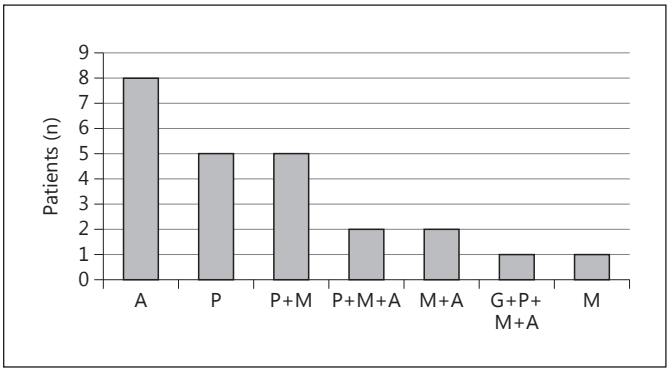

Fig. 1. ST results in patients with AMX as the culprit drug (n = 24). A = AMX; P = PPL; M = MDM; G = penicillin G.

Although patients who had a negative reaction to the AMX component tended to be younger than patients with a positive reaction, this difference did not reach statistical significance (15.4 ± 17.1 and 24.6 ± 27 years, respectively; p = 0.2). No difference was found in the demographic or clinical data between patients with and without a positive ST reaction to AMX, including the time interval between the allergic reaction and the test (table 1).

No adverse events were recorded except for a 15-year-old patient whose ST was positive only to AMX. She developed systemic pruritus accompanied by a few urticarial lesions immediately following ST with the higher con-

centration after ST with the lower concentration had been negative. Intramuscular epinephrine and oral antihistamine resulted in the resolution of all symptoms within minutes.

Discussion

Over 70 years, the optimal protocol to safely evaluate the large number of individuals with a history of an unconfirmed penicillin allergy for an active, clinically significant, IgE-mediated penicillin allergy, is still controversial. The oral AMX challenge under observation is considered the gold standard test [12]. Skin testing is essentially done to minimize the number of serious oral challenge reactions, yet still not overdiagnose penicillin allergy. In the past, it was believed that a high proportion of patients with immediate allergy to penicillin will react to the major component of penicillin (PPL) in a ST [13, 14]. A small proportion of these patients responded to other penicillin metabolites defined as minor determinants. Over the years, studies have demonstrated that combining PPL with minor determinants is appropriate for the evaluation of patients suspected of having immediate allergy to BL [15, 16]. Nevertheless, recent data suggest that the shift from using the classic benzylpenicillin antibiotic towards the new semisynthetic penicillins, especially AMX and AMX-C, has made PPL no longer the most relevant hapten in the evaluation of penicillin allergy [17]. Accumulating evidence suggests that a specific selective reaction to the side chains of AMX and AMX-C is the more important immunological mechanism than the cross-reactivity to the common BL structure [17–19]. Using semisynthetic penicillins such as AMX or AMX-C when testing those patients with suspected allergy to BL has become common practice in several centers, especially in Europe [7, 9, 20]. However, some centers, especially in the USA, still recommend the use of PPL without semisynthetic penicillins [10, 11].

In the present study, we specifically targeted the very small subgroup of individuals with a positive history of penicillin allergy to the semisynthetic penicillins, AMX and AMX-C, and a positive penicillin ST. First, we observed that more than half of the patients with immediate hypersensitivity reactions to BL reported either of these two antibiotics as the culprit drug. Moreover, over half of our patients had a positive reaction to the AMX component, and about 26% of the patients had a selective positive ST result only to this component. This finding is in concordance with results from previous publications [9, 21]. Furthermore, less than half of the patients responded to the major determinant PPL. Thus, using only PPL with the penicillin G determinant, as was recently suggested by Macy [10], would have resulted in a false negative ST result in more than half of our patients (54.9%). Although we do not know the clinical significance of these positive AMX or AMX-C ST results, because these patients did not undergo oral challenge, still, a false negative ST might jeopardize the patient with an anaphylactic, life-threatening reaction following OCT [12].

As for AMX-C specifically, the number of AMX-C-allergic patients was rather small. Nevertheless, only 2 out of 7 patients (28.5%) reacted to one of the 'classic' determinants, whereas most of these patients reacted only to either or both AMX and AMX-C. Once again, failure to use AMX or AMX-C upon testing this subpopulation would have led to a false negative result in 5 out of 7 patients (71.5%).

It has been suggested that the clinical presentation of penicillin-allergic subjects differs with regard to their immunologic response to major compared to minor determinants. Subjects with a positive ST to the major determinant tend to suffer from an urticarial rash after exposure to BL, whereas subjects with a positive ST to the minor determinants are reported to suffer more often from anaphylactic shock after exposure to BL [3]. As for the major determinant, it was speculated that this phenomenon was related to the development of IgG-blocking antibodies to benzylpenicillin, preventing the development of a systemic reaction [22]. In our study, there was no significant epidemiological or clinical difference between patients with a positive or negative AMX ST reaction. This is possibly due to the small sample size and the high percentage of urticaria as the clinical presenting sign in our subjects.

Side effects following ST with AMX and AMX-C mostly include urticarial rash, but rare cases of anaphylactic shock have also been described [23]. The reported rates of overall side effects after ST range from 5 to 11% of cases depending on the study [9, 10, 17, 24]. In the present study, only 1 patient (3.2%) whose ST was positive only to AMX developed a mild urticarial rash that disappeared immediately after treatment had been administered.

This study has few limitations. The first is the small number of patients. Nevertheless, our findings are consistent with those of others [3, 9, 21]. Second, we did not perform an OCT, which is the gold standard for confirming an immediate allergy diagnosis, due to safety and ethical reasons. This is in accordance with clinical guidelines and common practice [7, 12]. The potential reduction in

morbidity from positive oral challenges will still need to be compared to the known morbidity associated with an inaccurate penicillin allergy designation.

Third, we did not use the clavulanic acid kit (Diater), which is the preferred determinant for the evaluation of clavulanic acid allergy, because it was not available to us at the time the study was conducted. AMX-C used in skin testing is limited to a concentration of 20 mg/ml due to a possible irritant effect in higher concentrations, hence reducing the sensitivity of the test [25, 26].

In conclusion, the current study points out that using STs with the AMX and AMX-C determinants is mandatory in patients with a positive history of an immediate allergic reaction to these drugs. As these are, currently, the commonly used penicillins, failure to include them when performing penicillin skin testing may endanger patients with immediate reactions to BL if they are exposed to an unjustified OCT based on a false negative ST lacking these components.

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