ORIGINAL ARTICLE

Diagnosis of Patients With Immediate Hypersensitivity to ß-Lactams Using Retest

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Abstract

Background: ß-Lactams are the drugs most frequently involved in hypersensitivity reactions mediated by immunoglobulin (Ig) E.
Objective: To evaluate a population of patients with suspected ß-lactam allergy using a validated algorithm that includes specific IgE antibodies, skin testing, and/or a drug provocation test.
Methods: A total of 1032 patients with symptoms compatible with ß-lactam allergy were evaluated by means of their clinical history, specific immunoglobulin (Ig) E antibody determinations (benzylpenicillin, ampicillin, and amoxicillin), and skin tests with major determinants (penicilloyl-polylysine) and minor determinants (minor determinant mixture) of benzylpenicillin, penicillin G, ampicillin, and amoxicillin-clavulanic acid. Patients whose skin test results were negative were challenged with amoxicillin-clavulanic acid. Only immediate hypersensitivity reactions were evaluated. All patients with negative study results and for whom a reaction occurred more than 1 year before were retested using the same protocol.
Results: A total of 170 patients (16.4%) were finally confirmed as having immediate allergic reactions to ß-lactams (62.3% by skin testing, 16.5% by specific IgE, and 21.2% by drug provocation test). The mean age of these patients was 43.3 years, and the drug most frequently involved in the reaction was amoxicillin (41.1%), followed by the combination amoxicillin-clavulanic acid (36.4%). In the remaining 22.5%, different ß-lactams were involved or the culprit drug was not known. Only mild reactions were observed after the drug provocation test. A retest was required in 23% of patients in order to confirm their hypersensitivity.
Conclusions: These results indicate that a diagnostic protocol based on the combination of skin testing and in vitro determination of specific IgE antibodies plus, if required, drug provocation testing is an appropriate procedure for evaluating immediate hypersensitivity reactions to ß-lactams. Because the sensitivity of skin testing and in vitro IgE assays is not optimal and a considerable proportion of patients are tolerant, drug provocation tests are necessary to achieve the diagnosis or confirm tolerance. A large percentage of patients (23%) were diagnosed using retest.

Key words: Penicillins. Skin tests. Safety. Drug provocation test.

Resumen

Introducción: Los betalactámicos (BL) son los medicamentos más frecuentemente implicados en las reacciones de hipersensibilidad IgE mediadas.
Objetivo: evaluar una población de sujetos con sospecha de alergia a betalactámicos empleando un algoritmo ya validado, el cual incluye medición de IgE específica, Test Cutáneos (TC) y/o un Test de Provocación Controlada (TPC).
Métodos: Un total de 1032 pacientes con síntomas compatibles con alergia a betalactámicos fueron evaluados por historia clínica, IgE específica (bencilpenicilina (BP), ampicilina (AMP) y amoxicilina (AX)) y TC con los determinantes mayores (PPL) y menores (MDM) de BP, penicilina G, AMP y amoxicilina-clavulánico (AX–CLAV). Los pacientes con TC negativos fueron testados con AX-CLAV. Sólo se consideraron las reacciones de hipersensibilidad inmediata. Fueron retestados usando el mismo protocolo todos los pacientes con estudio negativo en el primer día de estudio y con última reacción hacía más de un año.
Resultados: Un total de 170 pacientes (16.4%) fueron diagnosticados de reacciones inmediatas con betalactámicos (62.3% por TC, 16.5% por IgE específica y 21.2% por TPC). La edad media de estos pacientes fue 43.3 años, y el medicamento más frecuentemente implicado fue AX(41.1%), seguido por AX-CLAV(36.4%). En el 22,5% restante, estuvieron implicados diferentes BLs o el medicamento causante no era conocido. Tras el TPC sólo se produjeron reacciones leves. Un 23% de los pacientes necesitó un retest para confirmar su hipersensibilidad.
Conclusiones: Estos resultados indican que este protocolo diagnóstico con una combinación de TC y medición de IgE específica in vitro y, si fuese necesario, TPC es un procedimiento necesario para evaluar reacciones de hipersensibilidad inmediata a betalactámicos. Al no ser la sensibilidad de los TC y de la IgE específica optima y un importante porcentaje de pacientes son tolerantes, el TPC es fundamental para descartar o confirmar hipersensibilidad. Un porcentaje importante de pacientes (23%) son diagnosticados usando retest.

Palabras clave: Penicilinas. Test cutáneos. Seguridad. Test de provocación controlada.

Introduction

In many allergology centers, drug allergy constitutes the third most frequent reason for consultation after rhinoconjunctivitis and bronchial asthma [1]. ß-Lactam hypersensitivity accounts for a very high proportion of cases of drug allergy evaluated by allergy departments. Based on the results of recent series and epidemiological studies from Spain [2], the frequency with which patients consult an allergologist in order to confirm a suspected drug allergy ranges from 12.63% to 14.7% of all allergy consultations. Of these, 47% of cases are attributed to reactions to ß-lactams.

Allergic reactions and other side effects of ß-lactams have increased in frequency since the 1950s, to the extent that they have become a public health issue [1,3]. However, many of the patients who consult for a suspected allergic reaction to ß-lactams [2,4] actually have good tolerance. Therefore, an accurate diagnosis is essential. The widespread use of these agents, a consequence of their substantially lower cost compared to other available antibiotics and their low toxicity, has resulted in ß-lactams becoming the drugs of choice (or even the sole or most advisable choice) for a large number of infectious diseases [5,6]. Patients consulting with a clinical history suggestive of ß-lactam allergy report a variety of symptoms, ranging from noncompatible reactions and mild allergic symptoms (widespread erythema, urticaria, or periorbital edema) to severe anaphylaxis (circulatory failure, cardiac and/or respiratory arrest, and death) [7,8]. However, information provided by the patient is very often inaccurate.

The diagnosis of hypersensitivity reactions to ß-lactams has become increasingly complex as a result of the growing number of available drugs and their widely varying chemical structures. For many years, the structure of the benzylpenicillin molecule conjugated to penicilloyl-polylysine (PPL) plus the minor determinant mixture (MDM; benzylpenicillin, benzylpenicilloic and others) was considered the classic basis for skin testing [8,9]. Additional determinants that consider the side chain structure must now also be taken into consideration [10,11]. The European Network on Drug Allergy (ENDA) of the European Academy of Allergy and Clinical Immunology (EAACI) recently designed a diagnostic algorithm for immediate reactions to ß-lactams [12]. The algorithm includes skin testing, in vitro testing, and direct provocation with the suspect drug (now indicated in up to 30% of patients) [10,13] and comes in 2 versions that can be used according to the facilities available in health centers. The short version enables patients to be diagnosed as having immediate reaction or not; the other provides a more detailed evaluation to determine whether patients are selective responders to specific side chain determinants or whether they recognize the common structure of penicillins [14].

We used the short algorithm to establish the role of the different diagnostic tests in the evaluation of patients with a history of hypersensitivity reaction to ß-lactams in our population [13,14]. The results show that drug provocation testing is necessary to confirm tolerance and/or establish a diagnosis in a large number of patients.

Material and Methods

Between January 2005 and December 2009, a total of 1032 patients with a suspected allergic reaction to ß-lactams were referred for diagnosis to the Allergy Department of Hospital Reina Sofía, Cordoba, Spain, a tertiary hospital covering a population of 800 000 inhabitants. Clinical records were completed according to the ENDA recommendations [8,14]. Data recorded included drug involved, time between drug intake and appearance of the reaction, time between the occurrence of the reaction and patient evaluation, type of symptoms induced, and number of episodes. Initially, these data enabled us to classify the reported reactions as allergic or nonallergic and according to severity in order to start prescribing the appropriate drug concentrations for skin testing [7,8,14,15]. All patients were informed about the potential risks and benefits of the study and signed the informed consent form in use at the time. The local ethics committee approved the study.

Due to the characteristics of our allergy department, we followed the short version of the ENDA diagnostic algorithm, with some modifications. If the suspected immediate reaction had occurred within 1 year before the study, we began with an in vitro test. If the in vitro test was negative, we continued with a skin test; if the skin test was negative, we performed a drug provocation test (Figure).

We compared the drug involved, the time between the last episode and the clinical study, and results in cases with a positive skin test result [16].

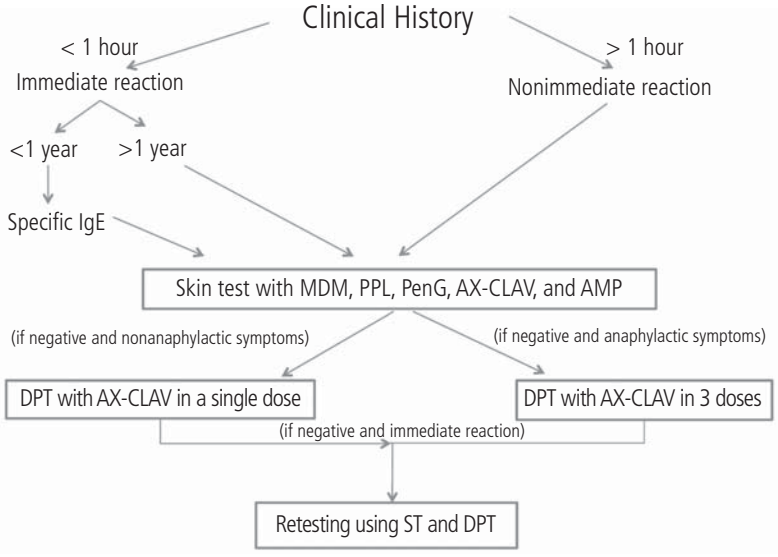

Figure. Diagnostic algorithm. AMP indicates ampicillin; AX, amoxicillin; CLAV, clavulanic acid; DPT, drug provocation test; Ig, immunoglobulin; MDM, minor determinant mixture; PenG, penicillin G; PPL, penicilloyl-polylysine; ST, skin testing.

In Vitro Assay

The in vitro assay was performed using a commercially available assay (ImmunoCAP System, Phadia, Uppsala, Sweden) with benzylpenicillin, amoxicillin, and ampicillin; values >0.35 kU_A/L were considered to reflect a positive result. Due to the decrease in sensitivity over time, this assay was only performed in those patients whose episode occurred during the previous year [14].

Skin Testing

Skin testing was carried out as previously described [14,15] using 0.02 mL of solution prepared daily. The reagents used were PPL (5×10^{-5}M), MDM ($2\times10^{-}$2M) (both provided by Diater, Madrid, Spain), benzylpenicillin (10 000 IU/mL: Ern Laboratories, Barcelona, Spain), ampicillin (20 mg/mL: Normon Laboratories, Madrid, Spain), and amoxicillin-clavulanic acid (20 mg/mL: Normon Laboratories, Madrid, Spain). The skin test results were considered positive or negative according to ENDA recommendations [14].

Drug Provocation Test

Drug provocation testing was performed in those cases with negative skin test and in vitro test results. Single-blind placebo-controlled challenge was carried out using amoxicillin-clavulanic acid in a single dose of 875/125 mg if the reaction was nonanaphylactic, and in 3 increasing doses (218/31, 437/62, 656/93) at 1-hour intervals if the reaction was anaphylactic, as described elsewhere [14,15]. The procedure was stopped when symptoms compatible with a hypersensitivity reaction appeared.

Retest

If the reaction had occurred more than 1 year before the study and negative results were obtained, we repeated the protocol 3 weeks later in order to evaluate the boosting capacity of the penicillin after the first evaluation and the potential conversion to a positive result (resensitization) [14,17,18,19].

Statistical Analysis

Qualitative variables, such as symptoms and number of positive results, were described using frequencies and percentages. Age and the time between the last adverse reaction and testing were reported as median (range). The statistical analysis was performed using the χ^2 test or the Wilcoxon–Mann-Whitney test as necessary.

Results

Of 1032 patients evaluated, 170 (16.47%) were confirmed as having a hypersensitivity reaction to ß-lactams. Mean age was 43.36 (8-84) years, and 110 (64.7%) patients were women ($P<.05$). The drugs involved in the reaction were amoxicillin in 70 patients (41.1%), amoxicillin-clavulanic acid in 62 (36.4%), unidentified penicillin in 36 (21.2%), and other ß-lactams in 2 (1.3%). Amoxicillin alone or in combination with clavulanic acid accounted for more than 70% of all cases, although this figure is probably greater if we also consider cases reporting an unidentified penicillin to have involved amoxicillin, as this drug was the most widely prescribed in the study population. The patients developed a mean of 1.40 (1-5) episodes, with cutaneous symptoms appearing in 106 patients (62.4%) and anaphylaxis in 64 (37.6%). The time between the reaction and the initial study was 89.71 (3-600) months (7.47 years), rising to 104.65 (5-195) months if we take into account the time at which patients were diagnosed by skin testing and to 106.79 (6-180) months for those diagnosed by drug provocation testing (differences not significant). The median time between drug intake and symptoms was 0.75 (0.083-0.9) hours.

Regarding the time interval between drug intake and start of the reaction, and according to the description given by the patients, all patients developed symptoms within 1 hour of taking the drug and were therefore considered to have had an immediate response.

From the total group of patients evaluated, 28 (16.5%) were diagnosed using the in vitro assay, 106 (62.3%) using skin tests, and 36 (21.2%) using drug provocation testing.

In Vitro Results

Of the 45 patients tested, the in vitro assay showed that 28 (62.2%) had a positive result (IgE levels >0.35 kU_A/L) to at least 1 penicillin determinant with 1 of the haptens used and were therefore considered to be allergic (no further studies were performed). Fourteen patients (32.14%) were positive for benzylpenicillin, 16 (39.28%) for ampicillin, and 17 (42.85%) for amoxicillin. No cases with nonimmediate reactions presented a positive in vitro result. No patients were positive to ampicillin only. Of all the patients with a positive result, 11 (39.29%) were positive for amoxicillin, 1 (3.57%) for ampicillin, and 1 for benzylpenicillin (3.57%) only. Nine patients (32.15%) were positive for benzylpenicillin and ampicillin and 4 patients (14.28%) for benzylpenicillin, ampicillin, and amoxicillin. In 2 patients (7.14%), the results for ampicillin and amoxicillin were positive.

Skin Testing Results

Skin testing was performed in those patients with a negative in vitro result. Of the total number of patients finally diagnosed as allergic to ß-lactams (N=170), 106 (62.3%) had positive skin test results.

On the first evaluation day, skin test results were positive to PPL in 20 patients (3 skin prick and 17 intradermal), to MDM in 16 (2 skin prick test and 14 intradermal test), to benzylpenicillin in 18 (3 skin prick and 15 intradermal), to ampicillin in 29 (7 skin prick and 22 intradermal), and to amoxicillin-clavulanic acid in 41 (11 skin prick and 30 intradermal). Table 1 shows the combinations detected in skin tests with the different haptens.

Positive skin test results to amoxicillin and ampicillin were more frequent in patients with a shorter interval since the last episode ($P<.05$) and if amoxicillin was the drug involved ($P<.05$). On the other hand, positive skin test results to benzylpenicillin determinants were observed in patients with a longer interval between occurrence of the reaction and

Table 1. Positive Results With Different Haptens in Patients Diagnosed in the First Evaluation and in Patients Diagnosed in the Retest

Reagents	Diagnosed in the First Evaluation (N=79)	Diagnosed in the Retest (N=27)
PPL	2 (2.53%)	3 (11.1%)
MDM	4 (5.06%)	2 (7.4%)
Penicillin G	4 (5.06%)	0
Ampicillin	9 (11.39%)	0
Amoxicillin-clavulanic acid	24 (30.37%)	6 (22.2%)
PPL and MDM	11 (13.92%)	2 (7.4%)
PPL and/or MDM and penicillin G	4 (5.06%)	0
Ampicillin and amoxicillin-clavulanic acid	19 (24.05%)	3 (11.1%)
Ampicillin and/or amoxicillin-clavulanic acid and penicillin G	10 (12.65%)	6 (22.2%)
PPL and/or MDM and/or ampicillin and/or amoxicillin-clavulanic acid	10 (12.65%)	2 (7.4%)
PPL and/or MDM and/or penicillin G and/or ampicillin and/or amoxicillin-clavulanic acid	16 (20.25%)	3 (11.1%)

Abbreviation: MDM, minor determinant mixture; PPL, penicilloyl-P-polylysine.

performance of the study ($P<.01$) and in those in whom an unknown penicillin was involved ($P<.05$).

Patients with a positive skin test result after the second evaluation are discussed below.

Drug Provocation Test

Drug provocation testing was performed with oral amoxicillin-clavulanic acid in those cases with negative in vitro results and skin test results. The total number of patients tested was 898, of whom 23 had positive results.

Patients reacted to cumulative doses of amoxicillin-clavulanic acid, as follows: 18 (78.3%) to 875/125 mg and 2 (8.7%) to 218.75/31.25 mg; and 1 (4.3%) to 437.5/62.5 mg and 2 (8.7%) to 656.25/93.75 mg. Thirteen patients (56.5%) presented cutaneous symptoms and 10 patients (43.5%) presented mild anaphylaxis, both of which conditions were controlled with medical treatment. The direct provocation test results are shown in Table 2.

Reevaluation of Cases With Negative Diagnostic Test Results

According to the protocol, all cases with a suggestive history but negative in vivo and in vitro results were reevaluated by repeating the same skin test and drug provocation test schedule. In the reevaluation, the diagnosis was confirmed in 40 cases: 27 by skin test and 13 by challenge. Regarding skin testing, 6 patients (22.2%) were positive to amoxicillin, 3 (11.1%) to PPL, and 2 (7.4%) to MDM only. Two patients (7.4%) were positive to PPL and MDM, 3 patients (11.1%) to amoxicillin and ampicillin, and 11 patients (40.8%) to either penicillin structure and side chain determinants. The results are shown in Table 1.

A total of 13 patients underwent retesting by drug provocation. Six patients presented cutaneous symptoms only, and 7 patients presented mild anaphylactic symptoms. All the reactions occurred within 1 hour after the provocation. The results are shown in Table 2. All the reactions were mild and well controlled within 60 minutes by intravenous methylprednisolone and antihistamines, with only 3 patients (23.07%) needing intramuscular adrenaline. No patients had to remain under observation for more than 2 hours. No biphasic reactions were seen.

The total number of positive results in the second evaluation—27 cases with skin tests and 13 cases with provocation—represent 23% of the total cases diagnosed as positive. Of the total number of challenges that were positive in all 36 cases, 13 responded in the second evaluation, showing that 7.6% of all cases diagnosed were positive.

Table 2. Clinical Symptoms During the Direct Provocation Test and Treatment Administered to Patients Diagnosed in the First Evaluation and Patients Diagnosed in the Retest

	#	Generalized Pruritus	Pharyngeal Pruritus	Urticaria	Angioedema	Maculo-papular Exanthema	Plantar Pruritus	Mild Dyspnea	Adrenaline
Diagnosed in the first evaluation	23	7	6	2	3	0	4	2	9
Diagnosed in the Retest	13	2	5	0	2	2	1	1	3

Discussion

Given the burden on the National Health System, patients with ß-lactam allergy are often studied long after their reactions have occurred.

During the 5-year study period, we evaluated a large number of patients with suspected allergic reaction to ß-lactams (N=1032). A diagnosis of allergy was established in 16.4% of the cases evaluated. These findings are similar to those reported by other groups, which showed that only 1 out of 4 patients or fewer is finally classified as allergic [10,13,20], indicating that most patients with a history of hypersensitivity reaction to ß-lactams tolerate the drug. Therefore, an accurate diagnosis is essential; otherwise, patients may be considered allergic and prescribed antibiotics that are more expensive and/or less efficient [5,11,13].

The general characteristics of our population are similar to those previously reported by other groups in terms of the ß-lactam involved [10,13,20-22]. In fact, the ß-lactams most frequently implicated in the reaction were amoxicillin and amoxicillin-clavulanic acid; consequently, we decided to use amoxicillin-clavulanic acid instead of amoxicillin alone for skin testing and drug provocation testing. The combination was used for skin testing, because clavulanic acid alone is not available for routine performance of skin testing [21,23]. It therefore follows that if there were any positive results to clavulanic acid, the optimal concentration used for skin testing was not achieved. A recent study indicated that the optimal concentration was 20 mg/mL [21], and this was not obtained with the combination of amoxicillin-clavulanic acid used.

The results obtained with the different diagnostic methods are also consistent with previous data [10,13], with 16.4% of patients diagnosed by in vitro assay, 62.4% diagnosed by skin testing, and 21.2% diagnosed by drug provocation testing. The results of skin testing were positive in less than two-thirds of patients. These data indicate that sensitivity is not optimal in cases of immediate reactions, and that as many as 30% of patients require an additional test to confirm the diagnosis, as published elsewhere [10,13].

The contribution of the in vitro IgE assay to identification of an immediate response was low (16.4%). This finding is similar to those of other studies [24], indicating that the value of this test is limited, possibly because of the use of a suboptimal system for quantification and the long interval between the occurrence of the reaction and the clinical study. A significant reduction has been observed in both basophil activation and specific IgE antibodies as the time interval between the occurrence of the reaction and the evaluation increases, to the extent that results become negative [25]. In fact, in our study, this interval was longer in those cases with negative IgE values than in those with positive values. Other studies have shown greater sensitivity of the in vitro test using commercially available options, although findings were based on a better selection of positive cases [26].

Many cases required a controlled challenge to establish the diagnosis. Our findings are consistent with those of other studies showing that neither skin testing nor in vitro IgE determinations enable a diagnosis to be established [10,13]. This concerns not only responses to benzylpenicillin, where the major/minor determinants of the antibiotic are considered classic determinants [7,10], but also specific side-chain determinants such as amoxicillin [27].

The number of patients who are resensitized after skin tests and/or provocation is relevant, because in some instances patients with a clear positive history but negative test results (including drug provocation testing) are recommended to undergo a second evaluation. Different studies indicate that 1% to 16% of patients may become sensitized after readministration of a ß-lactam [17-19,28,29]. In our study, these figures were higher; in fact, 25% of the patients diagnosed by skin testing had positive results only in the retest, although the results were negative at the first evaluation [18,19]. Of this total, 70% became positive to amoxicillin. These data are consistent with previous results [30], indicating that in vivo and in vitro sensitivity to amoxicillin decrease faster in patients sensitized to amoxicillin than in those who have positive results to classic benzylpenicillin determinants [16] and that patients are therefore more prone to a boosting effect [31]. As for ampicillin, 10 cases became positive. However, in no cases did ampicillin alone induce a positive response, indicating that when this drug did give a positive result, it was either by cross-reactivity with benzylpenicillin or with amoxicillin. The study by Romano et al [32] indicated that ampicillin might not be relevant for inclusion in a panel of penicillin determinants.

It is noteworthy that 9.3% of the provocation results became positive in the retest, indicating that a negative provocation result in a clear history is not enough to rule out the diagnosis and that optimal sensitivity cannot be achieved, even by using a complete battery of penicillin determinants including specific side-chain determinants.

Although amoxicillin was the most important drug involved, a significant proportion of patients remained positive to the benzylpenicillin determinants, PPL and/or MDM. This occurred even in those patients who, after being challenged with amoxicillin, had positive results with benzylpenicillin determinants in skin tests or challenges. Detailed in vitro studies using a radioallergosorbent test or radioallergosorbent inhibition assay have shown that, in some patients, after administration of amoxicillin (or even cephalosporins), the IgE antibodies recognized benzylpenicillin preferentially in the boosting response, indicating cross-reactivity between these structures [31].

This study has 2 limitations. First, the short algorithm did not enable discrimination between side-chain–specific reactors and cross-reactors to penicillins, because all the patients received amoxicillin-clavulanic acid only. This procedure was carried out with a protocol adapted to the limited resources available for evaluating patients with allergic drug reactions. Second, assessment with the amoxicillin-clavulanic acid obtained from the pharmaceutical vial used for skin testing is a limitation, because clavulanic acid has not been approved for in vivo diagnosis. This combination was also used in the drug provocation tests. Consequently, we were unable to verify whether patients were allergic to amoxicillin or to clavulanic acid, as published elsewhere [21,23].

In summary, we can conclude that, in addition to skin testing with a battery of penicillin determinants, drug provocation testing is also required for the diagnosis of patients

with immediate hypersensitivity to ß-lactams. Interestingly, 23% of cases were diagnosed after a second evaluation (skin testing and drug provocation testing), suggesting that a complete second evaluation needs to be carried out in order to confirm or rule out allergy to ß-lactams.

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