

# Basophil Histamine Release Induced by Amoxicilloyl-poly-L-lysine Compared With Amoxicillin in Patients With IgE-Mediated Allergic Reactions to Amoxicillin

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## ■ Abstract

*Background:* Amoxicillin (AX) is the  $\beta$ -lactam most often involved in IgE-mediated reactions. Diagnosis is based mainly on skin testing, although sensitivity is not optimal. We produced a new AX derivative, amoxicilloyl-poly-L-lysine (APL), and analyzed its recognition of IgE using the passive histamine release test (pHRT).

*Methods:* The study population comprised patients (n=19) with confirmed AX allergy and specific IgE to AX and controls (n=10) with good tolerance to AX. pHRT was performed using "IgE-stripped" blood from a single donor that was sensitized in vitro by patient sera and incubated with AX or APL. Histamine release was determined and expressed as nanograms of histamine released per milliliter of blood.

*Results:* The clinical symptoms were anaphylaxis (n=9), urticaria (n=7), erythema (n=2), and nondefined immediate reactions (n=1). The median (IQR) time interval between reaction and study was 90 (60-240) days and between drug intake and development of symptoms 24 (10-60) minutes. The median sIgE level was 3.37 (0.95-5.89) kU<sub>a</sub>/L. The sensitivity of pHRT to APL was 79% and the specificity 100%, which were higher than data obtained with pHRT to AX (63% sensitivity and 90% specificity). There was a positive correlation between maximal histamine release levels obtained with AX and APL (r=0.63).

*Conclusions:* In patients with immediate hypersensitivity reactions to AX, APL showed higher sensitivity and specificity than the culprit drug, AX, when tested in vitro by pHRT. This indicates that APL can improve the in vitro diagnostic accuracy of allergic reactions to AX. Further assessment of skin testing is necessary.

**Key words:** Allergy. Amoxicillin. Amoxicilloyl-poly-L-lysine. Basophil histamine release. Immediate reactions.

## ■ Resumen

*Introducción:* La amoxicilina (AX) es la betalactama más frecuentemente implicada en reacciones mediadas por IgE y el diagnóstico se basa principalmente en pruebas cutáneas (PC) aunque no tiene una óptima sensibilidad. Hemos diseñado un nuevo derivado de AX, amoxicilloyl-poly-L-lisina (APL), y se ha analizado el reconocimiento frente a la IgE mediante la prueba de liberación de histamina por sensibilización pasiva (pHRT).

**Métodos:** Se incluyeron pacientes (N = 19) con alergia a AX confirmada y con IgE específica positiva para AX y controles (N = 10) con buena tolerancia a AX. La pHRT se realizó utilizando sangre "libre de IgE" de un único donante, sensibilizada in vitro con el suero de los pacientes e incubada con AX o APL. La histamina liberada se determinó y se expresó como ng de liberación de histamina / ml de sangre. **Resultados:** Los síntomas clínicos de los pacientes fueron anafilaxia (N = 9), urticaria (N = 7), eritema (N = 2) y reacciones inmediatas no definidas (N = 1). El tiempo medio del intervalo entre la reacción y el diagnóstico fue de 90 días (IQR: 60-240) y entre la ingesta de fármacos y el desarrollo de los síntomas 24 min (IQR: 10-60). Los valores medios de sIgE fueron de 3.37 kU<sub>A</sub>/L (IQR: 0.95-5.89). La sensibilidad de pHRT a APL fue de 79% y la especificidad de 100%, siendo superior a la obtenida con pHRT a AX (63% de sensibilidad y 90% de especificidad). Hubo una correlación positiva entre los niveles máximos de liberación de histamina obtenidos con AX y APL (r = 0,63). **Conclusiones:** En los pacientes con reacciones de hipersensibilidad inmediata al AX, el APL mostró una mayor sensibilidad y especificidad en comparación con el fármaco causante de la reacción (AX), cuando se analizó por pHRT. Esto indica que APL puede mejorar la precisión diagnóstica in vitro de las reacciones alérgicas AX. Es necesario seguir utilizando PC.

**Palabras clave:** Alergia. Amoxicilina. Amoxiciloyl-poli-L-lisina. Basófilo liberación de histamina. Reacciones inmediatas.

## Introduction

$\beta$ -Lactam (BL) antibiotics are still the most frequent cause of IgE-mediated allergic reactions [1-3]. Although all BLs can be responsible, amoxicillin (AX) alone or combined with clavulanic acid and, to lesser extent, cephalosporins, are currently the most frequent culprits [1-3]. AX can induce selective reactions, implying that patients react to this antibiotic while tolerating other BLs such as benzylpenicillin (BP) [4-6], with immunochemical studies indicating that the side chain structure is important for immunological recognition [7-9].

The diagnosis of immediate allergic reactions to AX is based mainly on skin tests or drug provocation tests (DPT), procedures that are time-consuming and not risk-free [10-14]. Skin testing is performed using major and minor determinants of benzylpenicillin and AX, although its sensitivity is less than 70% [11]. The minor determinants of AX, amoxicilloyl and diketopiperazine [15], have been used to increase the sensitivity of skin testing, although neither is more sensitive than AX itself, indicating that new AX structures need to be investigated. Given that benzylpenicilloyl-octa-L-lysine was recently shown to be a stable compound that was useful for skin testing [16], it is tempting to speculate that amoxicilloyl-octa-L-lysine (APL) could be synthesized in a similar way.

In vitro tests, such as ImmunoCAP and the basophil activation test (BAT) are complementary approaches for the diagnosis of allergy to BL [17-20], although the former has low sensitivity [21] and the latter must be performed a few hours after blood extraction by expert personnel [19]. Other cellular tests, such as the passive histamine release test (pHRT), have proven useful for diagnosing clavulanic acid allergy [22]. pHRT is based on the detection of histamine release using "IgE-stripped" donor blood sensitized with patient serum followed by incubation with the antibiotic. It is suitable as a routine diagnostic test, since the plates can be sent to a reference laboratory for histamine detection and data analysis [23].

In this study, we produced a new synthetic product, APL, which it is expected to be more stable than the parent drug, AX. Basophil reactivity to both APL and AX was analyzed using pHRT with sera from patients with confirmed immediate allergic reactions to AX. This could be the first step in further evaluation of APL as a useful reagent for diagnosis based on skin testing.

## Material and Methods

### Patient Selection

The study group included patients diagnosed with immediate allergic reactions to AX in the allergy units of 4 Spanish hospitals during 2013. Diagnosis was based on the European Academy of Allergy and Clinical Immunology (EAACI) general guidelines for the evaluation of immediate reactions to BLs [12].

To be included, patients had to have experienced an allergic reaction within 90 minutes after administration of AX and have serum specific IgE (sIgE) to AX ( $\geq 0.35$  kU<sub>A</sub>/L). The control group comprised 10 healthy individuals who had previously received AX with no adverse effects, including allergic reactions, and with negative skin test and serum sIgE test results to AX.

The relevant institutional review boards approved the study, and all patients and controls gave their informed consent to participate.

### Skin Testing

Skin prick and intradermal tests were carried out as previously described [12], using 0.03 mL of solution prepared daily. The reagents were as follows: benzylpenicilloyl octa-L-lysine (BPOL) 0.04 mg/mL, with a molar concentration of the benzylpenicilloyl (BPO) moiety of  $8.64 \cdot 10^{-5}$  M; minor determinant (MD) 0.5 mg/mL, with a molar concentration of the sodium benzylpenilloate of  $1.5 \cdot 10^{-3}$  M; and AX 20 mg/mL (Diater Laboratories). In skin prick testing, a wheal larger than 3 mm surrounded by erythema with a negative response to control saline was considered positive. In intradermal tests, the wheal area was marked initially and 20 minutes after testing, and an increase in diameter greater than 3 mm surrounded by erythema was considered positive. In those cases with a history of severe anaphylaxis, we performed the test with increasing dilutions before reaching the maximum concentration.

### Specific IgE Determination

Specific IgE was determined using the CAP-FEIA system (Pharmacia Diagnostics) with c6 (amoxicilloyl) following the manufacturer's instructions (21). The results were obtained by

direct comparison with standards run in parallel, considering a value greater than 0.35 kU<sub>A</sub>/L to be positive.

### Synthesis and Characterization of APL

APL, a molecule with a molecular mass of 3966.6 Da and chemical formula C<sub>17</sub>H<sub>250</sub>N<sub>40</sub>O<sub>49</sub>S<sub>8</sub>, was obtained by clogging octa L-lysine to create a linear chain molecule with 8 recognizable fragments of amoxicilloyl. A schematic representation of the reaction and the resulting structure is shown in Supplementary Figure 1A. Briefly, sodium AX (in excess) and octa-L-lysine were dissolved in aqueous basic pH solution until no traces of octa-L-lysine were detectable. Acidification of the medium to pH <4.5 with HCl solution was followed by precipitation. The product obtained was prepurified with resuspension/centrifugation cycles in cold methanol, and the resulting solid mixture was lyophilized. The last purification stage was run through semipreparative chromatography using UV detection, selecting the APL eluting time and discarding the other residues. The resulting product was characterized by proton and carbon nuclear magnetic resonance (<sup>1</sup>H-NMR and <sup>13</sup>C-NMR) performed in a Bruker AV-300 spectrometer, as well as by Fourier transform infrared (FT-IR) spectrometry using a Nicolet Avatar 320 FT-IR spectrometer. The purity of the compound was analyzed using high-performance liquid chromatography (Supplementary Figure 1B).

### Passive Basophil Histamine Release Test

The pHRT was performed as previously described [22]. Briefly, passive sensitization of basophils was performed using heparinized blood from the same single healthy nonallergic donor, with a confirmed strong reaction to anti-IgE (histamine released, 45 [5] ng/mL) and no response to AX or APL. To remove the IgE bound on the surface of the basophils, 3 mL of blood sample was treated with 10 mL of stripping buffer (0.14 M NaCl, 0.005 M KCl, and 0.0134 M lactic acid) for 10 minutes at 4°C. Then, 0.5 mL of sera from patients or controls was incubated with 3 mL of the "IgE-stripped" donor blood for 60 minutes at 37°C. Aliquots (25 µL) of passively sensitized blood in the presence of IL-3 (2 ng/mL blood) were incubated in the glass fiber-prepared microtiter plates (RefLab ApS) with 25 µL of AX 5 µg/mL or APL 10 µg/mL for 60 minutes at 37°C. The AX and APL concentration chosen for this test was the highest value that did not lead to nonspecific histamine release (>10 ng/mL) in a dose-response curve. This level corresponded to the mean plus 3 standard deviations (SD) of the histamine release values measured in 5 nonallergic persons without a specific stimulus. As a positive control, we used 25 µL of anti-IgE (KPL) at a concentration of 5 µg/mL, as well as histamine at concentrations of 0 and 50 ng/mL.

After incubation, 75 µL of NaOH/ortho-phthalaldehyde (OPA) mixture was added to each well, allowing glass fiber-bound histamine to be released and coupled to OPA. After 10 minutes, the coupling reaction was stopped, and histamine-OPA complexes were stabilized by adding 75 µL of 0.59% HClO<sub>4</sub>. Histamine concentrations were determined in a Histareader 501 fluorometer (RefLab) and expressed as ng/mL of histamine released.

### Statistical Analysis

Quantitative variables were described with the median (IQR). Nonnormally distributed variables were compared using the Mann-Whitney test. Receiver operating characteristic curve analyses were performed to calculate the optimal cut-off value corresponding to the best sensitivity and specificity. All reported *P* values were 2-tailed, with values <.05 considered statistically significant.

### Results

APL was synthesized by acylation of the primary amino groups of octa-L-lysine with the β-lactam ring of the AX molecules. The chemical characterization of the resulting conjugate was based on <sup>1</sup>H-NMR and <sup>13</sup>C-NMR, whose spectra showed the signals corresponding to their structure (Supplementary Figure 1A and B). Characterization was also confirmed by FT-IR spectroscopy, which showed the absorption bands to be consistent with the functional groups included in the structure of the molecule.

The study included 19 patients with immediate hypersensitivity reactions to AX with the presence of serum sIgE to AX. The clinical characteristics and the results of the allergological work-up are shown in the Table. The median (IQR) age was 51 (33-62) years, 6 patients were female, and AX was the drug involved in all reactions. Nine patients developed anaphylaxis, 7 urticaria, and 2 erythema accompanied by intense pruritus; in 1 patient, the clinical reaction, although immediate, was not clearly defined. The median time interval between the reaction and the study was 90 (60-240) days and between drug intake and development of symptoms 24 (10-60) minutes. The median sIgE level to AX was 3.37 (0.95-5.89) kU<sub>A</sub>/L.

The ST results with AX were positive in the immediate reading in 10 patients: 7 had a positive prick test result at the maximum concentration of 20 mg/mL and 3 had a positive intradermal test result also at the maximum concentration of 20 mg/mL. The ST results were negative with AX in 2 patients, although they had a positive intradermal test result with BPOL (patient 10) or with BPOL and MD (patient 11). ST was not performed in 5 patients (4 anaphylaxis and 1 urticaria) owing to patient risk factors (patients 17 and 19) or because of the severity of the reaction (patients 1, 5, and 14). Finally, intradermal testing was not performed in 2 cases with a negative prick result to AX owing to the high positivity in the CAP assay (6.03 kU<sub>A</sub>/L in patient 8 and 5.26 kU<sub>A</sub>/L in patient 12). No patient developed systemic symptoms after ST, with negative results to BPOL, MD, and AX in all 10 controls.

For standardization of pHRT, we first performed a dose-response study to establish the highest concentration of AX and APL that did not induce nonspecific histamine release corresponding to 3+SD of background values measured in 5 nonallergic participants. We found that 5 µg/mL of either AX or APL were the best concentrations to perform the test and were therefore used for further studies (Supplementary Figure 2).

The pHRT results obtained with AX and APL are shown for each patient in the Table. The results for AX were significantly higher in patients (median, 16.5 [7-22] ng/mL) than in controls

Table 1. Clinical, Demographic Characteristics and Results of Skin Tests and Specific IgE to AX in the Study Population

Pat	Age, y	Gender	Reaction	IgE-AXO, kU <sub>A</sub> /L	IntStu, d	IntReac, min	Skin Test-AX, mm		HRT, ng/mL <sup>a</sup>	
							Prick	ID	AX	APL
1	24	Female	Anaphylaxis	1.14	90	5	ND	ND	15 <sup>a</sup>	17.5 <sup>a</sup>
2	31	Male	Urticaria	3.37	450	5	Pos (6)	ND	19.5 <sup>a</sup>	21.5 <sup>a</sup>
3	57	Male	Anaphylaxis	2.24	60	10	Pos (8)	ND	18 <sup>a</sup>	22 <sup>a</sup>
4	36	Female	Anaphylaxis	0.95	90	15	ND	Pos (11)	27 <sup>a</sup>	26 <sup>a</sup>
5	33	Female	Anaphylaxis	1.28	60	18	ND	ND	21 <sup>a</sup>	18.5 <sup>a</sup>
6	22	Male	Urticaria	1.65	180	10	Pos (6)	ND	30 <sup>a</sup>	23.5 <sup>a</sup>
7	64	Male	Erythema	3.57	90	30	Neg	Pos (10)	27.5 <sup>a</sup>	25 <sup>a</sup>
8	49	Male	Urticaria	6.03	210	60	Neg	ND	27.5 <sup>a</sup>	24.5 <sup>a</sup>
9	53	Male	Anaphylaxis	5.97	14	90	Pos (5)	ND	22 <sup>a</sup>	17.5 <sup>a</sup>
10	66	Male	Urticaria	>100	10	55	Neg	Neg <sup>b</sup>	21.5	19.5
11	56	Male	Urticaria	7.03	15	60	Neg	Neg <sup>c</sup>	12.5	17.5 <sup>a</sup>
12	35	Male	Anaphylaxis	5.26	45	30	Neg	ND	17.5 <sup>a</sup>	22.5 <sup>a</sup>
13	69	Male	NK	3.76	10680	NK	Neg	Pos (9)	15 <sup>a</sup>	22 <sup>a</sup>
14	47	Male	Anaphylaxis	0.85	240	30	ND	ND	8	13 <sup>a</sup>
15	51	Female	Erythema	5.89	90	60	Pos (8)	ND	7	4
16	28	Female	Urticaria	0.84	240	60	Pos (6)	ND	4.5	11
17	57	Male	Urticaria	0.81	180	10	ND	ND	6	5.5
18	62	Male	Anaphylaxis	0.76	60	5	Pos (7)	ND	5.5	3
19	74	Female	Anaphylaxis	3.86	240	10	ND	ND	3	24.5 <sup>a</sup>
1	45	Male	-	0.03	-	-	Neg	Neg	5	3
2	48	Female	-	0.07	-	-	Neg	Neg	10	9
3	37	Female	-	0.01	-	-	ND	ND	10	6
4	32	Male	-	0.01	-	-	Neg	ND	7	5
5	23	Male	-	0.03	-	-	Neg	Neg	6	6
6	33	Female	-	0.10	-	-	ND	ND	2	2
7	47	Female	-	0.01	-	-	Neg	Neg	6	4
8	28	Male	-	0.01	-	-	Neg	ND	7	7
9	44	Female	-	0.03	-	-	Neg	ND	5	1
10	47	Female	-	0.04	-	-	Neg	Neg	4	3

Abbreviations: APL, amoxicilloyl-octa-L-lysine; AX, amoxicillin; AXO, amoxicilloyl; HRT, histamine release test; ID, intradermal test; IntReac, time between drug administration and appearance of the symptoms; IntStu, time interval between reaction occurrence and the study; Neg, negative; NK, not known; ND, not done; Pos, positive.

<sup>a</sup>Positive results.

<sup>b</sup>ID positive to BPOL.

<sup>c</sup>ID positive to BPOL and DM.

(median, 12 [8.88-12.5] ng/mL). The results were also higher in patients for APL (median, 19.5 [13-23.5] ng/mL) than in controls (median, 8 [5.13-11] ng/mL). Comparison of the median values of histamine released between patients and controls showed differences using either AX ( $P=.13$ ) or APL ( $P=.0013$ ), with differences greater for APL (11.5 ng/mL) than for AX (4.5 ng/mL) (Figure 1). Although median histamine release with the APL molecule was higher than that obtained with AX, comparisons were not statistically significant (Figure 1). Moreover, a positive correlation was detected

between histamine release induced by AX and that induced by APL (Pearson  $r=0.631$  and  $P=.004$ ) (Figure 2).

Receiver operating characteristic curve analysis was performed to determine the optimal cut-off value for considering results as positive, with results indicating that values >12.5 ng/mL of histamine were the optimal cut-off for APL and >14.5 ng/mL of histamine for AX (Supplementary Figure 3). Using these cut offs, pHRT with APL revealed 15 out of 19 (79%) positive cases, and pHRT with AX revealed 12 out of 19 (63%) positive cases, thus indicating a higher

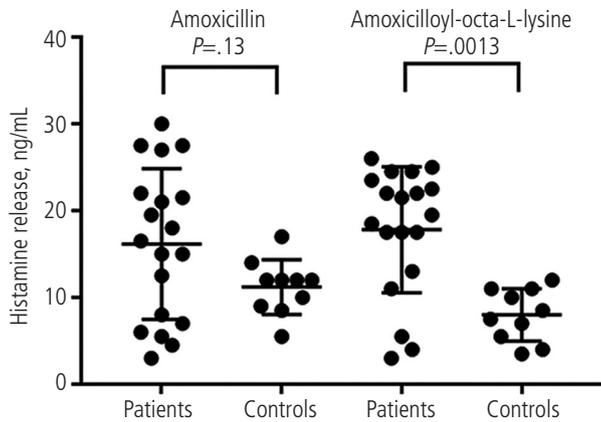


Figure 1. Comparison of results of the basophil passive histamine release (pHRT) test for AX and APL between patients and controls.

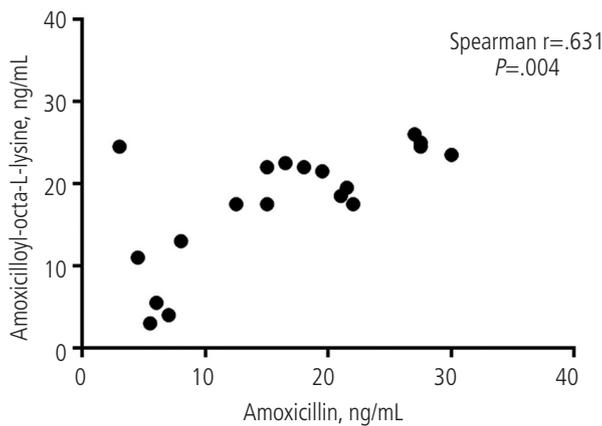


Figure 2. Correlation analysis between amoxicillin and APL in the basophil histamine release technique of allergic patients.

sensitivity for APL. The specificity results were also better for APL (100%) than for AX (80%). No patients responded to AX only.

## Discussion

Allergy to BLs is a major health problem, and 10% of the population report being allergic, although less than 20% are truly allergic [1,2]. Diagnosis is complex and based mainly on ST, with sensitivity less than 70%, thus implying that DPT is necessary for an accurate diagnosis in a nonnegligible percentage of cases [10-12,14]. The sensitivity of in vitro tests is not optimal and is especially lower with the CAP immunoassay [21,22]. The BAT with the highest sensitivity has the disadvantage that it requires fresh blood. Since pHRT was recently shown to be a useful method for diagnosing patients with immediate allergic reactions to clavulanate with a sensitivity of 55% and specificity of 85%, we applied this methodology in the present study [23].

All BLs used in clinical practice can induce allergy, although AX, whether combined or not with clavulanate, is the most common elicitor [1]. AX is a low-molecular-weight molecule formed by a  $\beta$ -lactam ring fused to a thiazolidine ring and a side chain [9]. According to the hapten hypothesis, AX needs to bind covalently to a carrier, usually a protein, in order to produce an immune response; this occurs through the opening of the  $\beta$ -lactam ring by the amino groups of protein lysine residues forming the amoxicilloyl antigenic determinant [25-27]. However, other structures such as amoxicilloic acid (which results from  $\beta$ -lactam ring hydrolysis) and diketopiperazine (which results from intramolecular acylation by the amino group of the AX side chain) can be formed. These structures have not proven useful in the diagnosis of immediate hypersensitivity reactions using the BAT [16].

The major determinant of BP, BPOL, is a stable product. A prospective multicenter clinical trial showed it to be useful for diagnosing patients with immediate BL allergy using ST [17]. Following the same synthetic approach, we produced APL by acylation of the primary amino groups of octa-L-lysine with the  $\beta$ -lactam ring of AX molecules. We used both APL and AX as reagents in pHRT in order to confirm specific recognition by sIgE from allergic patients bound to basophils. We chose pHRT instead of BAT for a number of reasons: (i) It is a simple method that has been improved using glass microfiber plates to which histamine is specifically bound and that enables the tests to be performed in any laboratory independently of the availability of equipment and the need to send them to a reference laboratory; (ii) Up to 10 drugs can be tested on the same plate; (iii) Given the possibility of in vitro determination, the test can be performed only by sending sera to centers where the technology is available. However, it is important to use a single donor with highly responding basophils in order to reduce interindividual variability due to basophil releasability.

Using this method, we obtained a sensitivity of 63% with AX and 79% with APL, and all patients with positive results with AX also had positive results with APL. In addition, specificity was higher with APL (100%) than with AX (80%). We also found a good correlation between positive in vitro histamine release responses to AX and APL, thus indicating that APL is a good reagent for in vitro diagnosis in patients with immediate hypersensitivity reactions to AX, especially if we take into account that we found positive results in 2 cases with negative ST results (Patients 10 and 11) and in 3 cases where ST was not performed (Patients 1, 5, and 19) owing to the severity of the reactions or the high risk for patients. In fact, in the 9 patients with negative ST results to AX, 8 (88.9%) had positive pHRT results with APL and 5 (55.5%) with AX, thus confirming the utility of this test for diagnosis.

Of note, all patients were selected based on a positive CAP result; therefore, pHRT had lower sensitivity for both AX and APL. However, we do not know the sensitivity of either method when compared in a general population with a history of immediate reaction to AX.

In conclusion, we show that pHRT using APL is a promising in vitro method for the evaluation of patients with immediate allergic reactions to AX, including patients in whom ST is negative or cannot be performed. Moreover, it would be

interesting to analyze the use of APL as a diagnostic reagent in both BAT and ST.

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### Conflicts of Interest

Sidsel Falkenchrone and Per Stahl Skov are research consultants for RefLab. Francisca Arribas, David Rodríguez, and Fernando Pineda are employees of DIATER Laboratories.

### References

- Dona I, Blanca-Lopez N, Torres MJ, Garcia-Campos J, Garcia-Nunez I, Gomez F, et al. Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. *J Investig Allergol Clin Immunol*. 2012;22(5):363-71.
- Torres MJ, Blanca M. The complex clinical picture of beta-lactam hypersensitivity: penicillins, cephalosporins, monobactams, carbapenems, and clavams. *Med Clin North Am*. 2010;94(4):805-20, xii.
- Romano A, Gaeta F, Valluzzi RL, Maggioletti M, Zaffiro A, Caruso C, et al. IgE-mediated hypersensitivity to cephalosporins: Cross-reactivity and tolerability of alternative cephalosporins. *J Allergy Clin Immunol*. 2015;136(3):685-91 e683.
- Bousquet PJ, Pipet A, Bousquet-Rouanet L, Demoly P. Oral challenges are needed in the diagnosis of beta-lactam hypersensitivity. *Clin Exp Allergy*. 2008;38(1):185-90.
- Blanca-Lopez N, Perez-Alzate D, Ruano F, Garcimartin M, de la Torre V, Mayorga C, et al. Selective immediate responders to amoxicillin and clavulanic acid tolerate penicillin derivative administration after confirming the diagnosis. *Allergy*. 2015;70(8):1013-9.
- Zambonino MA, Corzo JL, Muñoz C, Requena G, Ariza A, Mayorga C, et al. Diagnostic evaluation of hypersensitivity reactions to beta-lactam antibiotics in a large population of children. *Pediatr Allergy Immunol*. 2014;25(1):80-7.
- Miranda A, Blanca M, Vega JM, Moreno F, Carmona MJ, Garcia JJ, et al. Cross-reactivity between a penicillin and a cephalosporin with the same side chain. *J Allergy Clin Immunol*. 1996;98(3):671-7.
- Mayorga C, Obispo T, Jimeno L, Blanca M, Moscoso del Prado J, Carreira J, et al. Epitope mapping of beta-lactam antibiotics with the use of monoclonal antibodies. *Toxicology*. 1995;97:225-34.
- Ariza A, Mayorga C, Salas M, Dona I, Martin-Serrano A, Perez-Inestrosa E, et al. The influence of the carrier molecule on amoxicillin recognition by specific IgE in patients with immediate hypersensitivity reactions to betalactams. *Sci Rep*. 2016;6:35113.
- Fernandez TD, Mayorga C, Salas M, Barrionuevo E, Posadas T, Ariza A, et al. Evolution of diagnostic approaches in betalactam hypersensitivity. *Expert Rev Clin Pharmacol*. 2017;10(6):671-83.
- Torres MJ, Romano A, Mayorga C, Moya MC, Guzman AE, Reche M, et al. Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. *Allergy*. 2001;56(9):850-6.
- Torres MJ, Blanca M, Fernandez J, Romano A, Weck A, Aberer W, et al. Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy*. 2003;58(10):961-72.
- Torres MJ, Romano A, Celik G, Demoly P, Khan DA, Macy E, et al. Approach to the diagnosis of drug hypersensitivity reactions: similarities and differences between Europe and North America. *Clin Transl Allergy*. 2017;7:7.
- Gaeta F, Torres MJ, Valluzzi RL, Caruso C, Mayorga C, Romano A. Diagnosing beta-Lactam Hypersensitivity. *Curr Pharm Des*. 2016;22(45):6803-13.
- Lacombe-Barrios J, Salas M, Gomez F, Dona I, Ariza A, Mayorga C, et al. The Addition of Benzylpenicillin Does Not Increase the Skin Test Sensitivity Obtained With Classic beta-Lactam Determinants. *J Investig Allergol Clin Immunol*. 2016;26(1):52-4.
- Torres MJ, Ariza A, Fernandez J, Moreno E, Laguna JJ, Montanez MI, et al. Role of minor determinants of amoxicillin in the diagnosis of immediate allergic reactions to amoxicillin. *Allergy*. 2010;65(5):590-6.
- Fernandez J, Torres MJ, Campos J, Arribas-Poves F, Blanca M, Group DA-D. Prospective, multicenter clinical trial to validate new products for skin tests in the diagnosis of allergy to penicillin. *J Investig Allergol Clin Immunol*. 2013;23(6):398-408.
- Sanz ML, Gamboa PM, Antepará I, Uasuf C, Vila L, Garcia-Aviles C, et al. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy*. 2002;32(2):277-86.
- Torres MJ, Padial A, Mayorga C, Fernandez T, Sanchez-Sabate E, Cornejo-Garcia JA, et al. The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. *Clin Exp Allergy*. 2004;34(11):1768-75.
- Torres MJ, Ariza A, Mayorga C, Dona I, Blanca-Lopez N, Rondon C, et al. Clavulanic acid can be the component in amoxicillin-clavulanic acid responsible for immediate hypersensitivity reactions. *J Allergy Clin Immunol*. 2010;125(2):502-5 e502.
- Mayorga C, Celik G, Rouzaire P, Whitaker P, Bonadonna P, Cernadas JR, et al. In vitro tests for Drug Hypersensitivity

- Reactions. An ENDA/EAACI Drug Allergy Interest Group Position Paper. *Allergy*. 2016;71(8):1103-34.
22. Blanca M, Mayorga C, Torres MJ, Reche M, Moya C, Rodriguez JL, et al. Clinical evaluation of Pharmacia CAP System™ RAST FEIA amoxicilloyl and benzylpenicilloyl in patients with penicillin allergy. *Allergy*. 2001;56(9):862-70.
  23. Pineda F, Ariza A, Mayorga C, Arribas F, Gonzalez-Mendiola R, Blanca-Lopez N, et al. Role of Histamine Release Test for the Evaluation of Patients with Immediate Hypersensitivity Reactions to Clavulanic Acid. *Int Arch Allergy Immunol*. 2015;168(4):233-40.
  24. Kleine Budde I, de Heer PG, van der Zee JS, Aalberse RC. The stripped basophil histamine release bioassay as a tool for the detection of allergen-specific IgE in serum. *Int Arch Allergy Immunol*. 2001;126(4):277-85.
  25. Ariza A, Garzon D, Abánades DR, de los Ríos V, Vistoli G, Torres MJ, et al. Protein haptentation by amoxicillin: High resolution mass spectrometry analysis and identification of target proteins in serum. *J Proteomics*. 2012;77(0):504-20.
  26. Ariza A, Collado D, Vida Y, Montañez MI, Pérez-Inestrosa E, Blanca M, et al. Study of protein haptentation by amoxicillin through the use of a biotinylated antibiotic. *PLoS ONE*. 2014;9(3):e90891.
  27. Ariza A, Mayorga C, Fernandez TD, Barbero N, Martin-Serrano A, Perez-Sala D, et al. Hypersensitivity reactions to beta-lactams: relevance of hapten-protein conjugates. *J Investig Allergol Clin Immunol*. 2015;25(1):12-25.
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