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Clavulanic acid sensitization seems more involved in cutaneous than systemic reactions in amoxicillin-clavulanate drug reactions

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Key words

Clavulanic acid; amoxicillin; drug allergy; delayed reaction; systemic reaction.

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Impact statement

A significant percentage of mucocutaneous reactions after AX-CLA administration are due to CLA sensitization. In these cases, to not completely exclude AX, in vivo tests with clavulanate are useful.

Summary

Background. *Beta-lactams (BLs) allergy is considered a major health issue, as BLs are the most frequently involved in drug allergic reactions. Amoxicillin (AX) is the main sensitizer among all BLs. AX is commercialized alone or combined with clavulanic acid (CLA) in order to increase the antibiotic spectrum. The growing prescriptions of AX-CLA formulations contributed to increase the role of CLA as an allergy inducer. At present, little is known about the clinical characteristics of hypersensitivity reactions to clavulanate. The aim of this study was to assess the difference in the prevalence of cutaneous vs systemic reactions in patients with a documented history of allergic reactions to amoxicillin-clavulanate and tested positive for clavulanate or penicillin/amoxicillin.* ***Methods.*** *Between January 2017 and March 2023, out of 88 outpatients with suspected BLs allergy we selected 59 patients with a reaction to AX-CLA. Hypersensitivity reactions were classified according to onset time as immediate or delayed and according to clinical presentation as mucocutaneous or systemic reactions (anaphylaxis). All patients underwent recommended test protocols for diagnosing BLs hypersensitivity to identify the culprit drug. Sensitization was assessed through serologic and skin tests.* ***Results.*** *Patients whit immediate and delayed mucocutaneous reactions to AX-CLA are more sensitized to CLA 12/41 (29%) than AX or BLs determinants 9/41 (22%); on the opposite patients with immediate systemic reactions are more sensitized to AX or BLs determinants 13/18 (72%) than CLA 2/18 (11%), $p < 0.00$. There was no difference in immediate vs delayed reaction regarding CLA or AX and BLs determinants sensitization.* ***Conclusions.*** *Our study suggests that patients who presented only muco-cutaneous reactions were more often sensitized to CLA rather than AX.*

Introduction

Beta-lactams (BLs) are amongst the most commonly prescribed antibiotics in the community (1, 2) and the first choice for treating the majority of bacterial infections (3).
BLs allergy is considered a major health issue, as BLs are the most frequently involved in drug allergic reactions.
BLs hypersensitivity can be "immediate" or ''delayed" (4, 5). Immediate allergic reactions, ranging from cutaneous to systemic, usually appear within 1 hour, but may occur up to 6 hours after the last administered dose, and are mostly mediated by specific IgE antibodies (6).
Delayed reactions may occur at any time starting from 1 hour after drug administration, commonly after many days of treatment, and are often associated with a T-cell-dependent type of allergic mechanism (7, 8). Maculopapular exanthemas (MPE) and urticaria are the most common clinical features of delayed

reactions; less common presentations include fixed drug eruption and severe cutaneous adverse reactions (SCARs) (9-11).
Amoxicillin (AX) is the most frequently involved drug in sensitization among all BLs (6, 12). AX is commercialized alone or combined to clavulanic acid (CLA) in order to increase the antibiotic spectrum, as CLA inhibits bacterial beta-lactamases that nullify the effect of AX in resistant bacteria (13). Recent studies have shown that in younger people, AX-CLA is by far the most important drug triggering allergic reactions, accounting for up to 80% of BLs allergy cases (14). In the last years, the growing prescriptions of AX-CLA formulations contributed to increase the role of CLA as an allergy inducer (15, 16).
In the realm of literature, only a small number of allergic reactions associated with CLA have been documented. These reactions are predominantly attributed to type I immediate hypersensitivity, and to a lesser degree, delayed hypersensitivity (17-20). One of the significant challenges in this context is the constrained accessibility of skin testing for CLA and the absence of validated tests to measure serum-specific IgE (sIgE) levels in response to this drug (13).
At present, little is known about the clinical characteristics of hypersensitivity reactions to clavulanate.
The aim of this study was to assess the difference in the prevalence of cutaneous *vs* systemic reactions in patients who had a documented history of allergic reactions to amoxicillin-clavulanate and tested positive for clavulanate or penicillin/amoxicillin.

Materials and methods

We identified patients (n = 88) who were visited at our outpatient allergy department for an allergic reaction, immediate or delayed, after BLs intake (AX-CLA, amoxicilline, oxacilline) between January 2017 and March 2023, focusing on those who reported an adverse reaction to AX-CLA (n = 59).
Symptoms were collected from patients' medical records or clinical history. Hypersensitivity reactions were classified into four categories based on timing and clinical presentation: respectively immediate (within 1 hour up to 6 hours) *vs* delayed reactions (from 1 hour after the initial drug administration) and mucocutaneous *vs* systemic reactions.
Mucocutaneous reactions included urticaria, angioedema, generalized erythema, maculopapular exanthema and mucosal involvement. Systemic reactions included blood pressure drop related symptoms (*e.g.*, dizziness, fainting, need to lie down), wheezing, dyspnea, laryngeal edema, bronchospasm, dysphonia, dysphagia and all typical features of anaphylaxis.
All patients underwent serum specific IgE assay (ImmunoCAP®, Thermo-Fisher) for BLs (penicilloyl G, penicilloyl V, amoxicillin, ampicillin and cefaclor). In case of specific IgE positivity (cut off > 0.10 kUA/L), patients had their diagnostic process interrupted and they were challenged for alternative drugs instead.
Negative patients underwent skin prick test (SPT) and intradermal test (IDT).
Written informed consent was obtained from the patients to perform *in vivo* cutaneous tests and oral provocation tests.
Patients underwent SPT/IDT followed by OPT (5), using the following validated reagents provided by DIATER Laboratories (DAP; Madrid, Spain): benzylpenicilloyl-polylysine (PPL), minor determinant mixture (MDM), amoxicillin (20 mg/mL) and CLA (20 mg/mL). The maximum concentrations used were as follows: PPL 5 × 10-5 M, MDM 2 × 10-2 M, AX 20 mg/mL, and CLA 20 mg/mL.
The procedure was stopped when SPT or IDT at 15 minutes reading was positive. Patients were monitored for 2 hours after the last IDT.
Skin Tests (ST) were also evaluated at 48 h and 7 days to document delayed reactions. When negative, oral provocation test (OPT) was performed, according to a standardized BLs protocol (5). Patients with a positive clavulanate IDT, underwent OPT with amoxicillin 1,000 mg in a 3-days administration.
Data were tabulated using Excel 2020. Data are presented as frequencies of occurrence.
Comparisons between groups were performed 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

We examined a total of 59 adults (41 females, 18 males) reporting a hypersensitivity reaction (HR) temporally associated with AX-CLA. Patients' age ranged from 21 to 92 years (mean age: 54.22 years). 41 subjects (69%) reported cutaneous symptoms (13 immediate, 28 delayed) and 18 (31%) systemic symptoms (18 immediate, none delayed) (**table I**).

Mucocutaneous reactions

13/41 patients (32%) presented an immediate reaction, 28/41 (68%) presented a delayed reaction.
7 patients suffering from mucocutaneous reactions (17%) had a positive immunoCAP for BLs; 6 of them did not continue in ST procedure because of the high IgE levels and the possible risk of reaction. Only one patient with very low IgE levels continued in ST procedure (penicillin G and V specific IgE levels 0.29 and 0.60 kUA/L respectively, for a total IgE level 6428 kUA/L).
ST resulted as follow: 1) 12 patients showed IDT positivity to clavulanate (8 with an immediate reaction, 4 with a delayed positivity after 72 h); 2) 1 patient showed an immediate IDT positivity to PPL (ID 1:100), 2 showed a delayed IDT positivity to undiluted amoxicillin (20 mg/mL); 3) 20 patients were negative to skin tests and 6 patients did not perform ST because they all displayed an immunoCAP positivity to BLs.

Table I *- Types of reactions to AX-CLA and related sensitizations.*

Reaction	Symptoms	Positivity for PPL, MDM or AX (IgE assay and/or IDT)	IDT positive for CLA at immediate reading	IDT positive for CLA at delayed reading	Negative IgE assays for BBL and negative STs	Total
Immediate	Muco-cutaneous	6	3	0	4	13
	Systemic	13	1	1	3	18
Delayed	Muco-cutaneous	3	5	4	16	28
	Systemic	0	0	0	0	0
Total		**22**	**9**	**5**	**23**	**59**

Amongst patients experiencing a mucocutaneous delayed reaction (n = 28), 5 patients showed an immediate IDT positivity to clavulanate at 20 mg/mL, 4 showed a delayed IDT positivity for clavulanate at 20 mg/mL after 72 h, 1 showed a delayed IDT positivity to amoxicillin 20 mg/mL after 72 h, 16 showed negative ST, and 2 were not submitted to STs.

Systemic reactions

All patients (n = 18) presenting systemic symptoms had immediate reactions. 11 patients (61%) with systemic reactions displayed a positive immunoCAP for BLs. Only 2 patients with low specific IgE levels to BLs underwent ST because the reaction was reported in infancy.

ST performed in 9 patients resulted as follow: 1) 2 showed IDT positivity to clavulanate, of which 1 showed an immediate IDT positivity while the other one a delayed positivity after 72 h; 2) 4 showed an immediate IDT positivity to BLs (3 presented an IDT positivity to amoxicillin 20 mg/mL, 1 presented an IDT positivity to PPL and MDM 1:1), no one showed a delayed IDT positivity to BLs; 3) 3 had negative ST.

No patient resulted positive to both PPL/MDM or amoxicillin and clavulanate.

All the patients that showed an isolated clavulanate IDT positivity, both immediate and delayed, tolerated amoxicillin oral provocation challenge (**table II**).

No adverse events were recorded while performing ST and OPT.

Overall, patients who experienced mucocutaneous reactions after taking AX-CLA are more sensitized to clavulanate, while those who experienced systemic reactions are mainly sensitized to amoxicillin or BLs determinants. The result is statistically significant both considering the ST positivity alone ($p = 0.04$) or ST positivity and/or BLs ImmunoCAP positivity ($p < 0.00$).

No difference was found in the demographic data between patients with and without positive ST reaction to clavulanate.

There was no statistically significant difference in immediate *vs* delayed reaction regarding clavulanate or BLs test positivity.

Discussion and conclusions

Up to now, few studies focused on the different clinical characteristics of hypersensitivity reactions after AX-CLA administration. Our study suggests that patients who presented only muco-cutaneous reactions were more often sensitized to CLA rather than AX: AX-CLA hypersensitivity reactions probably differ according to the immunologic response either to clavulanate or to amoxicillin and BLs determinants. These data are in contrast with a recent Spanish study, reporting a high frequency (nearby 30%) of immediate systemic reactions in subjects sensitized to CLA (17). Since clavulanate seems to be a major culprit, in case of both immediate or delayed mucocutaneous reactions, our study suggests to test both CLA and BLs, in order not to exclude a BL as possible therapeutic strategy.

Our study does not demonstrate a statistical higher frequency of delayed rather than immediate reactions to AX-CLA in patients with delayed positive skin tests to clavulanate, probably due to a limited study population. Looking at the trend of this study anyway, a further implementation in diagnostic test to CLA will reach statistical significancy.

This study has some limitations: firstly, the little number of patients, partially due to COVID pandemic period; secondly, the lack of validated tests quantifying specific IgE to CLA. At the moment the only *in vitro* test to diagnose a CLA immunologic reaction is Basophil Activation Test (BAT), but this technology is limited to few laboratories, and not well standardized (21).

Table II *- Clinical characterics of patients with CLA skin test positivity.*

Sex	Age (years)	Timing of reaction	Reaction	Skin test	OPT
Male	30	30 minutes after AX-CLA (first dose)	Generalized urticaria	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	70	6th day of AX-CLA therapy	Generalized eritematous rash, pruritus	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	23	7th day of AX-CLA therapy	Maculo-papular rash	Delayed IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	20	6th day of AX-CLA therapy	Generalized urticaria	Delayed IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	23	24 hours after AX-CLA (first dose)	Maculo-papular rash, pruritus	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	56	8th day of AX-CLA therapy	Generalized urticaria	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	45	30 minutes after AX-CLA (first dose)	Urticaria, dyspnea, larynx edema	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Male	35	24 hours after AX-CLA (first dose)	Urticaria, dyspnea, nausea and vomiting	Delayed IDT positivity to CLA both 5 mg/mL and 20 mg/mL	Amoxicillin tolerated
Male	37	24 hours after AX-CLA (first dose)	Eritematous rash	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	34	4th day of AX-CLA therapy	Generalized urticaria	Delayed IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	62	5th day of AX-CLA therapy	Facial angioedema	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Male	63	2th day of AX-CLA therapy	Maculo-papular rash, pruritus	Delayed IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	82	60 minutes after AX-CLA (first dose)	Generalized urticaria	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	59	60 minutes after AX-CLA (first dose)	Generalized pruritus, edema of extremities	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated

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Contributions

FR, VP: conceptualization. CC, AS: writing – original draft, formal analysis. AF, VL: methodology. All authors: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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