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

Federica Rivolta [1], Camilla Cappelletti [2], Andrea Sangalli [2], Annalaura Fasiello [2], Valeria Longoni [2], Valerio Pravettoni[1]

[1] Department of Internal Medicine, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy
[2]Allergy and Clinical Immunology Residency, University of Milan, Milan, Italy

SUMMARY
BLs allergy is considered a major health issue, as BLs are the most frequently involved in drug allergic reactions. Amoxicillin (AX) is the most frequently involved drug in sensitization among all BLs. AX is commercialized alone or combined to 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 who had a documented history of allergic reactions to amoxicillin-clavulanate and tested positive for clavulanate or penicillin/amoxicillin. Our study suggests that patients who presented only muco-cutaneous reactions were more often sensitized to CLA rather than AX.

BACKGROUND
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) (9,10,11).
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 (17,18,19,20). 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.

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

Manuscript accepted for publication

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 (eg, dizziness, fainting, need to lie down), wheezing, dyspnoea, laryngeal oedema, 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 x 10-5 M, MDM 2 x 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 1000 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) (*see Table n.1*).

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: *(a)* 12 patients showed IDT positivity to clavulanate (8 with an immediate reaction, 4 with a delayed positivity after 72 h); *(b)* 1 patient showed an immediate IDT positivity to PPL (ID 1:100), 2 showed a delayed IDT positivity to undiluted amoxicillin (20 mg/mL), *(c)* 20 patients were negative to skin tests and 6 patients did not perform ST because they all displayed an immunoCAP positivity to BLs.
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: *(a)* 2 showed IDT positivity to clavulanate, of which 1 showed an immediate IDT positivity while the other one a delayed positivity after 72 h; *(b)* 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, *(c)* 3 had negative ST.

Manuscript accepted for publication

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.
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.

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).

FUNDING INFORMATION

This study was partially funded by Italian Ministry of Health, Current research IRCCS

CONFLICT OF INTEREST STATEMENT

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

AUTHOR CONTRIBUTIONS

FR and VP provided the patient data regarding outpatients clinical records and conceived the study; CC and AS wrote the manuscript and made contributions to interpretation of data; AF and VL organized the database. All authors critically read and approved the final manuscript.

References

1. Confino-Cohen R, Rosman Y, Lachover I, Meir Shafrir K, Goldberg A. The Importance of Amoxicillin and Amoxicillin-Clavulanate Determinants in the Diagnosis of Immediate Allergic Reactions to β-Lactams. Int Arch Allergy Immunol. 2016;170(1):62-66. doi:10.1159/000446961
2. Versporten A, Coenen S, Adriaenssens N, Muller A, Minalu G, Faes C et al. ESAC Project Group. European Surveillance of Antimicrobial Consumption (ESAC): outpatient penicillin use in Europe (1997-2009). J Antimicrob Chemother. 2011 Dec;66 Suppl 6:vi13-23. doi: 10.1093/jac/dkr454. PMID: 22096062.
3. Shenoy ES, Macy E, Rowe T, Blumenthal KG. Evaluation and Management of Penicillin Allergy: A Review. JAMA - J Am Med Assoc. 2019;321(2):188-199. doi:10.1001/jama.2018.19283
4. Torres MJ, Mayorga C, Pamies R, Rodriquez JL, Juarez C, Romano A et al. Immunologic response

Manuscript accepted for publication

to different determinants of benzylpenicillin, amoxicillin, and ampicillin. Comparison between urticaria and anaphylactic shock. Allergy. 1999 Sep;54(9):936-43. doi: 10.1034/j.1398-9995.1999.00175.x. PMID: 10505456.
5. Romano A, Atanaskovic-Markovic M, Barbaud A, Bircher AJ, Brockow K, Caubet JC, et al. Towards a more precise diagnosis of hypersensitivity to beta-lactams - an EAACI position paper. Allergy. 2020 Jun;75(6):1300-1315. doi: 10.1111/all.14122. PMID: 31749148.
6. Antúnez C, Martín E, Cornejo-García JA, Blanca-Lopez N, R-Pena R, Mayorga C et al. Immediate hypersensitivity reactions to penicillins and other betalactams. Curr Pharm Des. 2006;12(26):3327-33. doi: 10.2174/138161206778194042. PMID: 17017927.
7. Demoly P, Adkinson NF, Brockow K, Castells M, Chiriac AM, Greenberger PA et al. International Consensus on drug allergy. Allergy. 2014 Apr;69(4):420-37. doi: 10.1111/all.12350. PMID: 24697291.
8. Pichler WJ. Immune pathomechanism and classification of drug hypersensitivity. Allergy Eur J Allergy Clin Immunol. 2019;74(8):1457-1471. doi:10.1111/all.13765
9. Romano A, Viola M, Mondino C, Pettinato R, Di Fonso M, Papa G et al. Diagnosing nonimmediate reactions to penicillins by in vivo tests. Int Arch Allergy Immunol. 2002 Oct;129(2):169-74. doi: 10.1159/000065876. PMID: 12403935.
10. Romano A, Blanca M, Torres MJ, Bircher A, Aberer W, Brockow K et al; ENDA; EAACI. Diagnosis of nonimmediate reactions to beta-lactam antibiotics. Allergy. 2004 Nov;59(11):1153-60. doi: 10.1111/j.1398-9995.2004.00678.x. PMID: 15461594.
11. Park CS, Yang MS, Kang DY, Park HJ, Park SY, Nam YH et al; Drug Allergy Work Group of KAAACI. Risk factors of beta-lactam anaphylaxis in Korea: A 6-year multicenter retrospective adult case-control study. World Allergy Organ J. 2021 Sep 8;14(9):100580. doi: 10.1016/j.waojou.2021.100580. PMID: 34567348; PMCID: PMC8433252.
12. Blanca M, Vega JM, Garcia J, Miranda A, Carmona MJ, Juarez C et al. New aspects of allergic reactions to betalactams: crossreactions and unique specificities. Clin Exp Allergy. 1994 May;24(5):407-15. doi: 10.1111/j.1365-2222.1994.tb00928.x. PMID: 8087651.
13. Torres MJ, Montañez MI, Ariza A, Salas M, Fernandez TD, Barbero N et al. The role of IgE recognition in allergic reactions to amoxicillin and clavulanic acid. Clin Exp Allergy. 2016 Feb;46(2):264-74. doi: 10.1111/cea.12689. PMID: 26662186.
14. Kulhas Celik I, Guvenir H, Hurmuzlu S, Toyran M, Civelek E, Kocabas CN et al. The negative predictive value of 5-day drug provocation test in nonimmediate beta-lactam allergy in children. Ann Allergy Asthma Immunol. 2020 May;124(5):494-499. doi: 10.1016/j.anai.2019.12.029. Epub 2020 Jan 7. PMID: 31923549.
15. Fernandez-Rivas M, Carral CP, Cuevas M, Marti C, Moral A, Senent CJ. Selective allergic reactions to clavulanic acid. J Allergy Clin Immunol. 1995;95(3):748-750. doi:10.1016/S0091-6749(95)70181-8
16. Longo N, Gamboa PM, Gastaminza G, Audícana MT, Antepara I, Jaúregui I et al. Diagnosis of clavulanic acid allergy using basophil activation and leukotriene release by basophils. J Investig Allergol Clin Immunol. 2008;18(6):473-5. PMID: 19123441.
17. Torres-Rojas I, Pérez-Alzate D, Somoza ML, Pfeifer AP, Diaz EH, Jimenez-Rodriguez TW et al. Clavulanic Acid Is a Leading Culprit Beta-Lactam in Immediate Allergic Reactions to Penicillins. Allergy Asthma Immunol Res. 2023 Mar;15(2):201-213. doi: 10.4168/aair.2023.15.2.201. PMID: 37021506; PMCID: PMC10079519.
18. Calvão J, Batista R, Gonçalo M. Two cases of delayed hypersensitivity to clavulanic acid proven by patch tests. Contact Dermatitis. 2021;85(3):370-372. doi:10.1111/cod.13859
19. Conus S, Straumann A, Bettler E, Simon HU. Selective allergic reactions to clavulanic acid: A report of 9 cases. J Allergy Clin Immunol. 2010;126(1):175-177. doi:10.1016/j.jaci.2010.04.029
20. Mitchell L. Sensitization to clavulanic acid in Augmentin. Physiotherapy. 2002;79(8):583. doi:10.1016/s0031-9406(10)60312-4
21. Salas M, Fernández-Santamaría R, Mayorga C, Barrionuevo E, Ariza A, Posadas T et al. Use of the Basophil Activation Test May Reduce the Need for Drug Provocation in Amoxicillin-Clavulanic Allergy. J Allergy Clin Immunol Pract. 2018 May-Jun;6(3):1010-1018.e2. doi: 10.1016/j.jaip.2017.08.009. Epub 2017 Sep 28. PMID: 28964705.

Manuscript accepted for publication

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

Table 1. **MC symptoms** = Mucocutaneous symptoms

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*	*Eritematous rash*	*Immediate*	*Amoxicillin*

Manuscript accepted for publication

		(first dose)		*IDT positivity to CLA 20 mg/mL*	*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*

Table 2. Clinical characterics of patients with CLA skin test positivity (

Manuscript accepted for publication