

Delayed hypersensitivity associated with amoxicillin-clavulanate

To the Editor,

Beta-lactam/beta-lactamase combinations are prevalently used in hospital-acquired infections and prescribing data suggests that drugs such as amoxicillin-clavulanate are amongst the most commonly prescribed antibiotics in the community^{1,2}. Selective immediate reactions to clavulanate have been well described particularly from Southern Europe; however, little is known about selective delayed reactions.³ We report on a novel cohort of patients with a history of delayed reaction to amoxicillin-clavulanate who demonstrated a delayed intradermal skin test response to clavulanate.

Patients reporting a delayed amoxicillin-clavulanate allergy phenotype that completed beta-lactam skin prick (SPT) and intradermal testing (IDT) at the Drug and Antibiotic Allergy Services of Austin Health and Peter MacCallum Cancer Centre (VIC, Australia) between 1 May 2015 and 1 February 2019 were identified from a prospectively collected database. Patients underwent SPT/IDT followed by oral provocation as per a standardized previously published beta-lactam protocol, including validated Diater reagents (DAP; Madrid, Spain) which was used for the major (benzylpenicilloyl-poly-L-lysine [PPL]) and minor determinant mixtures (MDM) and clavulanate.⁴ In addition, IDT was performed to clavulanate (2 mg/mL or 5 mg/mL and 20 mg/mL) for selected patients (not routinely available at our service). A positive delayed IDT test was a >5 mm erythematous, raised and indurated or infiltrative lesion present at 6–48 hours post-IDT (at the site of IDT).⁵ Oral provocation in patients with a positive clavulanate intradermal test was undertaken with phenoxymethylpenicillin potassium (5-day provocation) and amoxicillin (5-day provocation). In patients with confirmed clavulanate hypersensitivity, peripheral blood mononuclear cells (PBMCs) were isolated from whole heparinized blood and stored at –80°C in 90% heat-inactivated foetal bovine serum (FBS) and 10% dimethyl sulfoxide until IFN- γ release enzyme-linked immunospot (ELISpot) assay analysis was performed as per previously published methods.⁶ The mean number of spots for the test and unstimulated wells was calculated. A positive response was defined as equal or greater to 50 spot forming unit (SFU)/million cells after background (unstimulated control) removal (dotted line) (Figure S1).⁷

From the prospective cohort of 1069 patients, we identified 66 (6.2%) patients reporting an adverse drug reaction (ADR) temporally associated with amoxicillin-clavulanate. Amongst these, 30 (45.5%) reported delayed hypersensitivity, 23 (34.8%)

immediate hypersensitivity and 13 (12.1%) a non-immune-mediated or unknown reaction. For the non-immune-mediated or unknown reactions, 11 (11/13; 84.6%) had the allergy label removed without testing. Concerning the patients with immediate amoxicillin-clavulanate hypersensitivity skin test positivity, 6 (26%) patients had positive skin testing to ampicillin and 2 (8.6%) to clavulanate. From the 30 patients with a reported delayed amoxicillin-clavulanate hypersensitivity, 18 (60%) underwent testing with clavulanate in addition to the routine beta-lactam protocol. Six (33.3%) patients were positive to clavulanate at either concentration on IDT (Table 1). For the six patients that tested positive to clavulanate, one was positive to both ampicillin and clavulanate (Table 1: ID 6). From those that had an isolated clavulanate IDT positive ($n = 5$), 4/5 tolerated amoxicillin and penicillin oral provocation and one (Table 1: ID 2) refused amoxicillin challenge but tolerated phenoxymethylpenicillin potassium and cefuroxime 5-day oral challenge. Overall, in those patients with an immune-mediated amoxicillin-clavulanate allergy history ($n = 53$), 6 (11.3%) were confirmed on clavulanate skin testing. An example of a positive skin test is demonstrated in Figure 1.

We found that two patients (33%) were positive (Table 1: ID 1, 2) to clavulanate on ELISpot testing (Figure S1) utilizing previously published criterion.⁶ One of the patients presented borderline positive response at 50 SFU/million cells and might reflect a false-positive result or low activated peripheral T-cell numbers. These findings are possibly related to the delay between the skin eruption and the allergy investigations. Also, new data demonstrate that resident memory T cells in the skin are likely to be a major player in the reproducibility of skin testing, where peripheral blood may be unreactive.⁸ Furthermore, we note that the amoxicillin-clavulanate ELISpot was negative in those with positive ELISpot to clavulanate. This may be related to a lower immunogenicity of amoxicillin-clavulanate or to the fact that this combination generates different haptenated proteins than clavulanate alone. The ELISpot results for the cohort are demonstrated in Supplementary materials (Figure S1).

Isolated clavulanate hypersensitivity has been reported in the literature. A Portuguese prospective cohort (7-year period) examined severe IgE-mediated hypersensitivity to clavulanate. In their cohort of 166 patients, they identified 6 (3.6%) cases of isolated immediate clavulanic acid allergy confirmed by either skin testing or positive oral challenge.³ In this specific cohort, the authors identified that selective allergy to clavulanate represented five of all immediate

TABLE 1 Baseline and clinical characteristics of patients with a positive delayed intradermal test to clavulanate

ID	Age/ Sex	ICH	Phenotype [∇]	RegiSCAR	Latency (months)	ID testing (mg/ml)	Positive IDT	Positive ELISpot ^a	Post-testing OC tolerated ^b
1	47/F	0	B4 (Severe MPE †)	2	18	Clav 5/20 Beta-lactam standard ^c	Clav 20	Yes	Amoxicillin Penicillin VK Cefuroxime
2	55/F	0	B4 (Severe MPE †)	2	35	Clav 5/20 Beta-lactam standard ^c	Clav 20	Yes	Penicillin, Cefuroxime
3	70/M	1	B4 (Severe MPE †)	3	2	Clav 5/20 Beta-lactam standard ^c	Clav 20	No	Amoxicillin Penicillin VK Cefuroxime
4	63/M	0	B4 (MPE)	1	2	Clav 5/20 Beta-lactam standard ^c	Clav 20	No	Amoxicillin Penicillin VK
5	66/F	1	B4 (MPE)	3	5	Clav 5/20 Beta-lactam standard ^c	Clav 20	No	Amoxicillin Penicillin VK Cephalexin
6	70/F	0	B4 (DRESS)	4	2	Clav 5/20 Beta-lactam standard ^c	Clav 20 Ampicillin	No	Cefuroxime Penicillin VK

Abbreviations: Clav, clavulanate; DRESS, drug reaction with eosinophilia and systemic symptoms; ELISpot, enzyme-linked immunospot assay (A positive response was defined as equal or greater than 50 SFU/million cells after background (unstimulated control) removal; ICH, immunocompromised; IDT, intradermal testing; MPE, maculopapular exanthema; OC, oral challenge; RegiSCAR, Registry of severe cutaneous adverse reaction diagnosis score for drug rash and eosinophilia with systemic symptoms (Final score: <2 = no case, 2-3 = possible case, 4-5 = probable case, > 5 = definitive case).

[∇] Adverse drug reactions are subclassified as type A and B reactions. Type B reactions correspond to drug hypersensitivity reactions. Type B1 includes the IgE-mediated reactions, B2, the antibody-mediated cytotoxicity reactions, B3, the immune complex-mediated reactions and type B4, all the delayed reactions.

† Severe MPE defined as an extensive cutaneous exanthema with more than 50% of body surface area and RegiSCAR score of 2-3 (possible).

Tested IDT concentrations: benzylpenicillin, 1,000 IU/mL; benzylpenicillin, 10,000 IU/mL; Diater PPL Neat, Diater MDM; ampicillin, 25 mg/mL; flucloxacillin, 2 mg/mL; cephazolin 1 mg/mL, ceftriaxone 2.5 mg/mL

^aELISpot testing was performed in all patients for amoxicillin, augmentin and clavulanic acid.

^bAll delayed challenges [5 days].

^cAs per previously published methods—patients underwent SPT/IDT followed by oral provocation as per a standardized previously published beta-lactam protocol, including validated Diater reagents (DAP; Madrid, Spain) used for the major (benzylpenicilloyl-poly-L-lysine [PPL]) and minor determinant mixtures (MDM) and clavulanate^{4,16}.

reactions to beta-lactams (N = 32) highlighting the need for clavulanate allergy assessment. Lezmi and colleagues identified, from a paediatric cohort, 11 cases of isolated delayed hypersensitivity to clavulanate based on positive prolonged amoxicillin-clavulanate provocation and associated negative amoxicillin challenges.^{9,10} In an adult population study summarizing results of penicillin allergy testing, five cases of isolated clavulanate allergy were described (intradermal clavulanate positive/amoxicillin challenge negative).¹⁰ In this cohort (N = 5), 80% of reactions were immediate allergy phenotypes. Finally, several case reports described isolated clavulanate delayed hypersensitivity of varied severity from mild skin eruptions¹¹ to acute generalized exanthematous pustulosis.^{11,12} There are limited reports of delayed clavulanate hypersensitivity, also confirmed by IFN- γ release enzyme-linked immunospot assay.

In summary, we identified that 3% of prospective antibiotic allergy-tested cohort reported a delayed amoxicillin-clavulanate hypersensitivity, 13% of which had a confirmed isolated clavulanate

hypersensitivity. Previous published reports have confirmed immediate clavulanate hypersensitivity^{1,13,14}; however, literature detailing cases of skin test or ex vivo diagnostic confirmed delayed hypersensitivity are uncommon. Further, infrequently reported in the literature is dual sensitization to amoxicillin and clavulanate,¹⁵ also demonstrated in a single patient in our cohort. Clinicians should be alert to patients reporting amoxicillin-clavulanate allergies and the potential for an isolated hypersensitivity, ensuring the beta-lactam “window” does not necessarily close on this patient cohort forever.

This study was approved by the Austin Health ethics committee, and the investigators obtained written informed consent from the participants.

KEYWORDS

allergy diagnosis, challenge tests, drug allergy, immunologic tests, pharmacology

FIGURE 1 A, Pictorial representation of a patient reporting delayed hypersensitivity to clavulanate from tested cohort. Maculo-papular erythematous fixed eruption. B, Photographic representation of positive delayed intra-dermal clavulanate testing (24 h postinoculation) in the same patient reporting a delayed hypersensitivity to amoxicillin clavulanate. Tested IDT concentrations: (a) normal saline; (b) 2 mg/mL clavulanate; and (c) 20 mg/mL clavulanate



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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supplementary Figure 1: ELISpot results

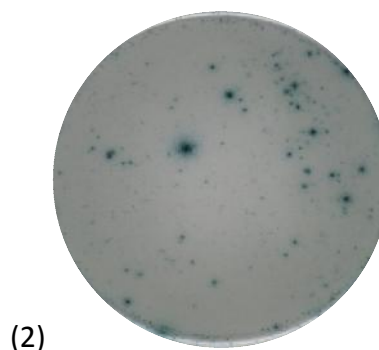
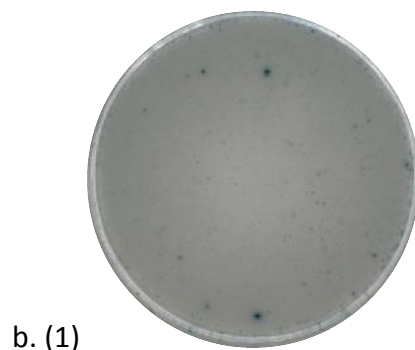
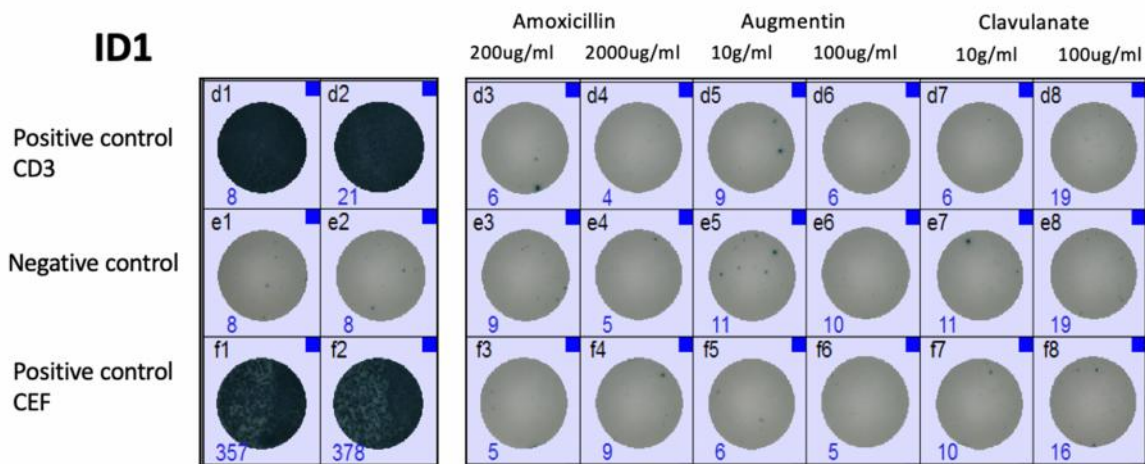
a. Positive IFN- γ release ELISpot for ID1 (patients with positive IDT to clavulanate and tolerance to oral amoxicillin).

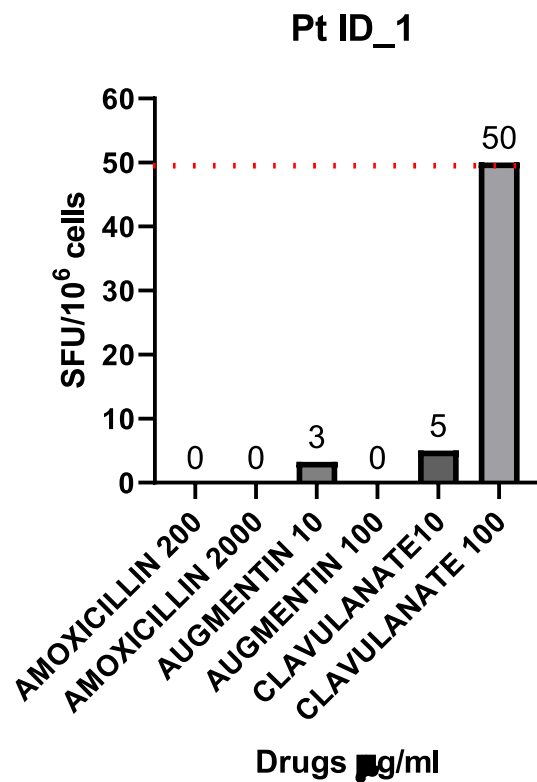
CEF (Cytomegalovirus, Epstein –Barr and Influenza) is the second positive control composed of a peptide pool of well- defined viral epitopes from Cytomegalovirus, Epstein –Barr and Influenza viruses

b. Well for Clavulanic acid 100 μ g/ml for ID 1(1) and ID2 (2).

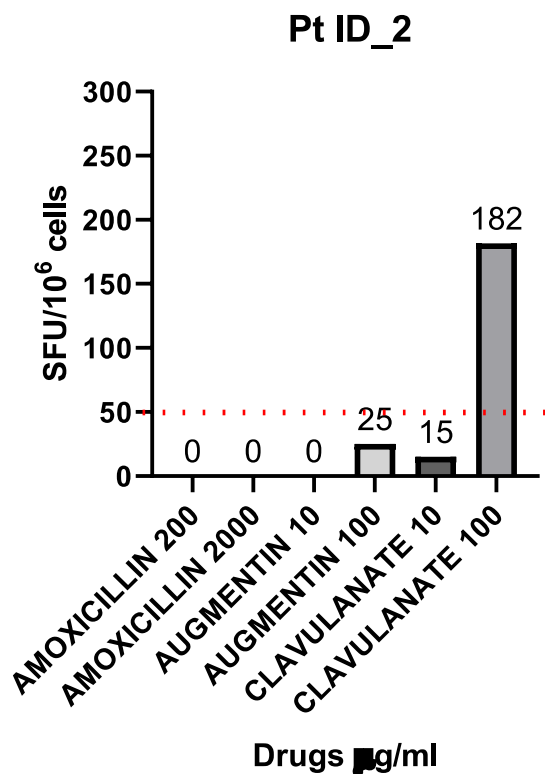
c. ELISpot graphical illustration for ID 1 (1) and ID2 (2). Positivity is defined by ≥ 50 spot forming unit (SFU)/million cells after background (unstimulated control) removal (dotted line).

a.





c. (1)



c.(2)