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Title: 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 1st May 2015 and 1st February 2019 were identified from a prospectively collected database. Patients underwent SPT/IDT followed by oral provocation as per a standardised previously published beta-lactam protocol, including validated Diater reagents (DAP; Madrid, Spain) which was used for the major (benzylpenicilloyl-poyl-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 to 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 -80C 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) (**Supplement Figure 1**) [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. Among 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 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 2 patients (33%) were positive (**Table 1: ID 1, 2**) to clavulanate on ELISpot testing (Supplement **Figure 1**) 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 demonstrates 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 (**Supplement Figure 1**).

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 5 of all immediate reactions to beta-lactams (N = 32) highlighting the need for clavulanate allergy assessment. Lezmi and colleagues identified, from a pediatric 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, 5 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 eruptions [11] 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.

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184 Dr. Trubiano has nothing to disclose.

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*	Post-testing OC tolerated†
1	47/F	0	B4 (Severe MPE ǂ)	2	18	Clav 5/20 Beta-lactam standard‡	Clav 20	**Yes**	Amoxicillin Penicillin VK Cefuroxime
2	55/F	0	B4 (Severe MPE ǂ)	2	35	Clav 5/20 Beta-lactam standard‡	Clav 20	**Yes**	Penicillin, Cefuroxime
3	70/M	1	B4 (Severe MPE ǂ)	3	2	Clav 5/20 Beta-lactam standard‡	Clav 20	No	Amoxicillin Penicillin VK Cefuroxime
4	63/M	0	B4 (MPE)	-1	2	Clav 5/20 Beta-lactam standard‡	Clav 20	No	Amoxicillin Penicillin VK
5	66/F	1	B4 (MPE)	-3	5	Clav 5/20 Beta-lactam standard‡	Clav 20	No	Amoxicillin Penicillin VK Cephalexin
6	70/F	0	B4 (DRESS)	4	2	Clav 5/20 Beta-lactam standard‡	Clav 20 Ampicillin	No	Cefuroxime Penicillin VK

Abbreviations: *ICH, immunocompromised; IDT, intradermal testing; OC, oral challenge; Clav, clavulanate; DRESS, drug reaction with eosinophilia and systemic symptoms; MPE, maculopapular exanthema; 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); ELISpot, enzyme linked immunospot assay (A positive response was defined as greater than 50 SFU/million cells after background (unstimulated control) removal.*

∇ 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 includes all the delayed reactions.

***** ElISpot testing was performed in all patients for Amoxicillin, Augmentin and Clavulanic acid

† All delayed challenges [5-day]

‡ As per previously published methods - Patients underwent SPT/IDT followed by oral provocation as per a standardised previously published beta-lactam protocol, including validated Diater reagents (DAP; Madrid, Spain) used for the major (benzylpenicilloyl-poyl-L-lysine [PPL]), minor determinant mixtures (MDM) and clavulanate[1,2].

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

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

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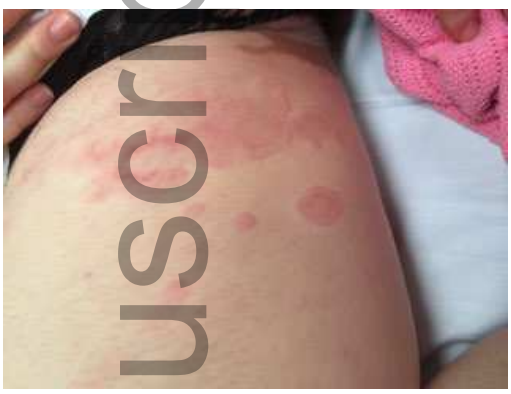
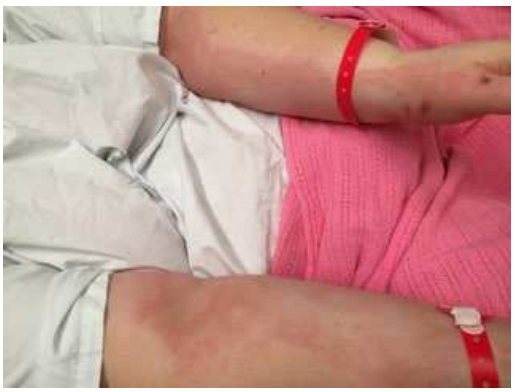
Figure 1: a. Pictorial representation of a patient reporting delayed hypersensitivity to Clavulanate from tested cohort. Maculo-papular erythematous fixed eruption

b. Photographic representation of positive delayed intra-dermal clavulanate testing (24 hours post-inoculation) in the same patient reporting a delayed hypersensitivity to amoxicillin clavulanate. Tested IDT concentrations: 1. Normal Saline; 2. 2mg/ml Clavulanate; 3. 20mg/ml Clavulanate

a.





b.