

TITLE:

Delayed positive drug provocation tests to betalactams with flare-up reactions of skin tests sites.

Running title

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Conflict of interest statement

None

Author contributions

- All authors gathered the cases, reviewed previous literature and contributed equally to the final manuscript.

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Clinical implication box: Although reactions elicited by drug provocation tests to penicillins usually appear within 48h of a one-day exposure to the drug, very delayed reactions (up to 9 days) may occur. Clinicians working in drug allergy should be aware of this peculiar delay and inform their patients, since these may be true drug allergies, confirmed by flare-up of primarily negative skin tests.

To the editor,

Non-immediate allergic drug hypersensitivity reactions (DHR) to betalactams are attributed to T cell-dependent mechanisms, varying from mild/moderate reactions, such as maculopapular exanthema (MPE) or delayed urticaria, to more severe cutaneous adverse reactions (SCAR)^[1]. Patch tests (PT) and/or delayed-reading intradermal tests (IDT) have been proposed for *in vivo* diagnosis of this allergic phenotype, with the latter being generally reported as more

sensitive ^[1]. When skin tests are negative and there is no contraindication, a drug provocation test (DPT) may be performed, to confirm or rule out the DHR. However, a positive DPT alone does not allow any insight into the mechanism of the confirmed DHR. In a previous analysis of DPT to BL, we have shown that in most cases, the reaction is elicited within 48 hours after the drug administration^[2].

Flare-up reactions consist in the reactivation of previous positive skin tests or the switch from negative to positive skin tests, usually after the performance of a systemic challenge (but also after repeated PT). The flare-up phenomenon has been known and described for contact eczema (e.g. nickel) for more than fifty years. Since then, it has been described for some drugs such as: ampicillin ^[3], amoxicillin (flare-up within 24 hours after DPT)^[4], trimethoprim–sulfamethoxazole^[5], Glatiramer acetate (GA)^[6], heparin^[7] and ibuprofen ^[8].

We describe four cases of very delayed positive DPT (6 to 9 days after one-day DPT) confirming non-immediate DHR to penicillins, associated with a flare-up phenomenon on IDT sites. All were female patients, with an initial reaction occurring more than ten years before testing. In all cases, we performed skin tests (prick and IDT) with immediate and when appropriate, delayed-reading (up to 7 days) for a panel of penicillins (penicillin G, amoxicillin +/- clavulanic acid, ampicillin) and an alternative betalactam, namely cefuroxime, according to the European recommendations at the time of testing^[1]. In case of negative skin tests, one-day DPT was performed, as previously described^[2]. In case of unspecified culprit betalactam, amoxicillin (+/-clavulanic acid) was chosen for DPT, due to its frequent use in France.

The patients' characteristics are detailed in Table 1.

Patient 1 described delayed urticaria as her initial reaction. Skin tests were negative. Nine days after the DPT, generalized MPE and eosinophilia occurred and IDT became positive on penicillin G and amoxicillin sites. There were no

signs of internal organ involvement. Regular complete blood counts were performed until the eosinophil count returned to normal (on the third week since the onset).

Patient 2 reported delayed facial angioedema and generalized urticaria as her initial reaction. Skin tests were negative. Six days after the DPT, MPE occurred and the patient had a flare-up on the IDT sites of amoxicillin and amoxicillin/clavulanic acid.

Our third patient had diarrhoea and MPE as her initial reaction, but she could not specify the delay of occurrence. The patient developed a MPE and eczematous lesions appeared on amoxicillin/clavulanic acid and ampicillin IDT sites 7 days after the DPT (Figure A).

The fourth patient described an initial reaction of MPE appearing 48h after cefatrizine (aminocephalosporin with a side chain identical to amoxicillin) intake. DPT to amoxicillin/clavulanic acid was performed 8 days after skin testing. She developed a generalized MPE 7 days after the DPT associated with eczema of amoxicillin/clavulanic acid and ampicillin IDT sites.

We present the largest case series of very delayed positive DPT to betalactams after a single day DP. The reactions elicited by DPT were in all cases MPE. No skin biopsies were performed in these patients, at the time of the positive DPT and therefore, immunohistochemical data is not available. Of note, in all of cases the reactions were associated with flare-up reactions on skin test sites, allowing to confirm true allergic DHR, *a posteriori*. Upon questioning the patients we were able to rule out other differential diagnosis (e.g., other drug intake or infection). If the clinical presentation supported by positive skin tests matches known phenotypes of non-immediate allergic DHR, the delay of occurrence is hardly common. In a previous analysis on DPT to betalactams from our group, over a 20-year period, we showed that all but two

patients (1.1%, Patient 1 and 4 in this series), reacted within 48 h after the last dose administered during DPT^[2]. Upon discharge after DPT, we systematically inform the patients with suspected DHR of non-immediate or unknown chronology of the possibility of delayed reactions. A medical prescription and an e-mail/phone contact are provided. Since our first analysis on the delay of occurrence of positive DPT, two more patients presented this very delayed profile, which can hardly be explained by the pharmacokinetics of amoxicillin (plasmatic half-life of 1-2h). After oral ingestion, tissue fixation of penicillin and its immune recognition is probably involved, but to the best of our knowledge, this data is not quantified for penicillin allergy. In all but one patient, the index clinical history had taken place more than three decades before the allergy work-up. We could speculate that the delayed reaction in DPT (6-9 days after the last intake) and the associated flare-up might be related to this large interval, as previously suggest⁵. Nevertheless, T-cell mediated response is known to last over decades. In three cases (Patients 1,2,3), DPT was performed on the same day of skin tests, and delayed reading was not deemed necessary because of the type of reaction in the initial clinical history, involving urticaria/angioedema or MPE with associated diarrhoea. The mechanisms of delayed urticaria are not well documented, and we did not expect to elicit positive skin tests (e.g., eczema) in delayed reading. Considering the reaction elicited by the DPT and on ST sites, we make the assumption that the index reactions in these patients were most likely non-immediate MPE, and not urticaria.

The histopathological mechanisms involved in the flare-up phenomenon are still unknown. Local skin memory in which persisting T cells are involved has been previously described. The resident T cells are CD4+ CCR10+, located in clusters around the venules, and are subject to homing induced by CCL27, whose expression remains increased for several weeks after the inflammation phase^[9]. The peculiarity in our case series is the long interval between DPT performance

and the flare-up phenomenon. For drugs, the published cases include flare-ups immediately after DPT or within maximum 24 hours. This phenomenon differs from the booster effect, which has been observed amongst immunocompromised patients. In this situation, a two-step tuberculin skin test procedure may be required to “boost” a potential immune memory against tuberculin that was waned over time, eliciting a false-negative result upon the first test but a recall response on repeat testing resulting in a positive reaction.

In conclusion, although reactions elicited by DPT to penicillins usually appear 48h of a one-day exposure to the drug, very delayed DHR may occur, as late as 9 days after the ingestion. Clinicians working in drug allergy should be aware of this unusual delay, since these may be true drug allergies, confirmed by flare-up of primarily negative skin tests.

Patient Nº, age at the time of testing	Time initial reaction / allergy work-up	Chronology and type of reaction	Drug involved	Severity signs on initial reaction	Skin test <i>Year of allergy work-up</i> <i>Delay to positive ST</i> <i>Drugs with positive ST</i>	DPT	Reaction after DPT
1, F, 34 years	25 years	Non-immediate; Day 8: Urticaria	Penicillin (unspecified)	None	2006 Day 9 Positive: Peni G, Ax	To Ax Performed the same day as IDT	Day 9: MPE and eosinophilia (990 cells/mm ³). Duration: 7 days for

							cutaneous signs, 3 weeks till normal eosinophil count, no sequellae. Treatment: topical corticosteroids, antiH1 and oral prednisolone 5 days.
2, F, 62 years	40 years	Non-immediate; Day 2: Facial angioedema and generalized urticaria Two episodes.	Penicillin (unspecified)	None	2017 Day 6 Positive: Ax, AxClav	To AxClav Performed the same day as IDT	Day 6: MPE on chest and back. Duration: 5 days. Treatment: topical corticosteroids, antiH1.
3, F, 63 years	50 years	Unknown timing; MPE + diarrhoea	Penicillin (unspecified)	Healing over 3 weeks	2017 Day 7 Positive AxClav, Ap	To AxClav Performed the same day as IDT	Day 7: Generalized MPE and eosinophilia (900 cells/mm ³). Duration: 15 days, no sequellae. Viral PCR (EBV, CMV, HHV6) negative. Treatment:

							typical corticosteroids, antiH1 and oral prednisolone for 5 days.
4, F, 20 years	18 years	Non-immediate; Day 2: Generalized MPE	Cefatrizine**	None	2015 Day 15 Positive: AxClav, Ap	To AxClav Performed 8 days after IDT	Day 7: Generalized MPE . Duration: 7 days, no sequellae. Viral pCR (EBV, CMV, HHV6): negative. Treatment: topical corticosteroids, anti-H1.

Table 1. Description of the four patients with delayed positive drug provocation tests to betalactams.

ST: skin test; **Peni G:** Penicilin G; **Ax:** Amoxicilin; **DPT:** drug provocation test; **IDT:** Intradermal testing; **MPE:** maculopapular exanthema; **AxClav:** Amoxicilin/Clavulanic acid; **Ap:** Ampicillin.

antiH1, antihistamine H1; **EBV:**Epstein Barr virus; **CM;**, cytomegalovirus; **HHV:-**herpes virus 6

** Cefatrizine could not be used for DPT in this patient due to its removal from the French market

References:

¹Torres MJ, Sanchez-Sabaté E, Alvarez J et al. Skin tests evaluation in non immediate allergic reactions to penicillins. *Allergy* 2004; 59:219–24.

²Chiriac AM, Rerkpattanapipat T, Bousquet PJ, Molinari N, Demoly P. Optimal step doses for drug provocation tests to prove beta-lactam hypersensitivity. *Allergy*. 2017 Apr;72(4):552-561.

³Reig Rincón de Arellano I, Villalón García AL, Cimarra Alvarez-Lovell M, Robledo Echarren T, Martínez-Cócera MC. Flare up to betalactams. *Allergol Immunopathol*. 2005;33:282-284.

⁴Tramontana M, Hansel K, Bianchi L, Agostinelli D, Stingeni L. Flare-up of previously negative patch test and intradermal test with amoxicillin after oral provocation. *Contact Dermatitis*. 2018;79:250–251.

⁵Kardaun SH, de Waard MM, de Monchy JG. Flare-up of patch test of trimethoprim-sulfamethoxazole (co-trimoxazole) during oral desensitization. *Contact Dermatitis*. 2009;61:50-51.

⁶Minciullo PL, Calapai G, Gangemi S. Flare up reaction during provocation test to glatiramer acetate in a patient with allergy to interferon beta1a. *Allergy Asthma Immunol Res*. 2014;6:467-469.

⁷García Robaina JC, Sánchez Machín I, Fernández-Caldas E, de la Torre Morín F. Delayed systemic reactions with flare-ups of previously negative intradermal skin tests to heparin. *Allergy*. 2003;58:685-686.

⁸González de Olano D, González Mancebo E, Gandolfo Cano M, Menéndez Baltanás A, Trujillo Trujillo MJ. Flare-up-like phenomenon in a skin prick test after oral challenge with ibuprofen. *J Investig Allergol Clin Immunol*. 2007;17:414-415.

⁹Moed H, Boorsma DM, Tensen CP, et al. Increased CCL27-CCR10 expression in allergic contact dermatitis: implications for local skin memory. *J Pathol*. 2004;204:39-46.





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