Cenk Suphioglu

NeuroAllergy Research Laboratory (NARL), School of Life and Environmental Science, Faculty of Science, Engineering and Built Environment, Deakin University, Waurn Ponds, Vic, Australia

Correspondence
Cenk Suphioglu, NeuroAllergy Research Laboratory (NARL), School of Life and Environmental Sciences (LES), Faculty of Science, Engineering and Built Environment (SEBE), Deakin University, Geelong Waurn Ponds Campus, 75 Pigdons Road, Geelong, VIC 3216, Australia.
Email: cenk.suphioglu@deakin.edu.au

ORCID
Cenk Suphioglu https://orcid.org/0000-0003-0101-0668

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SUPPORTING INFORMATION

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

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Sensitivity and specificity of lymphocyte transformation test in children with mild delayed hypersensitivity reactions to beta-lactams

To the Editor,

Beta-lactams (βLs) and, among them, amoxicillin (AMX) and amoxicillin-clavulanic acid (AMX-CL), are the most frequent causes of drug allergies. In children, clinical pictures are often delayed-type reactions ranging from mild maculopapular exanthemas (MPEs) in 90% of cases to life-threatening Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), or drug reaction with eosinophilia and systemic symptoms (DRESS).[1]

The diagnosis of true sensitization to βLs is based on a complex work-up with some critical issues because the clinical history is often unreliable, and the sensitivity of skin tests is not optimal. Thus, a drug provocation test (DPT) may be required to establish a correct diagnosis.[S1, S2]

This point is critical in delayed reactions to βLs, as the diagnostic strength of the allergy work-up is lower than in immediate reactions.[2]

Drug-reacting T cells are thought to be the key player in delayed-type hypersensitivity reactions (HRs). Thus, delayed-reading intradermal tests (IDTs), patch tests (PTs), and lymphocyte transformation test (LTT) have been proposed as the only diagnostic tools allowed after severe reactions. In delayed reactions of mild entity of children, several recent studies highlighted the importance of DPT, even skipping in vivo tests as poorly sensitive. Despite that DPT is considered the gold standard for HRs,[S2] there is no consensus on the duration time of the oral challenge. On the other hand, the added diagnostic value of in vitro tests, such as LTT, has been so far evaluated only in few papers in the pediatric population.[3]

Our aim was thus to evaluate how DPT correlates with LTT in delayed reactions to βLs assessing sensitivity, specificity, and the predictive value of this test to ascertain sensitization.

To this end, 50 children with positive histories of delayed skin reactions after AMX or AMX/CL were consecutively investigated with a complete allergy work-up.[S3] At hospital admission according to the hospital ethic Committee form, all the parents of the children undergoing the allergy work-up signed an informed consent to the processing of clinical data for future research studies. Patients whose parents denied consent were excluded from the study. All the children were analyzed twice. Firstly, clinical history was recorded according to the ENDA questionnaire[S1] and blood samples were collected to perform LTT. At a second evaluation, all the patients

TABLE 1 Clinical characteristics, in vivo and in vitro tests in DPT positive children

			Clinical history							
Pt. n°	Sex/age (yrs)	Culprit drug	Symptoms	Latency (hrs) [day of treatment]	PTs	SPT (wheal mm)	IDTs (wheal mm/erythema mm)	DPT Reaction [day]	Latency of DPT reaction (hrs)	LTT
#1A	F/7	AMX/CL	U	2 [3°]	NP	NEG	NEG	U [2°]	3	NEG
#2A	M/5	AMX/CL	U	Unknown	NP	NEG	NEG	MPE [3°]	Unknown	NEG
#3A	M/11	AMX/CL	ER	24 [6°]	NP	NEG	NEG	U [4°]	Unknown	POS
#4A	F/14	AMX/CL	U	12 [1°]	NP	NEG	NEG	GI, F, MPE [1°]	24	POS
#5A	F/9	AMX/CL	MPE	Unknown [3°]	NP	NEG	NEG	ER [1°] U [2°]	few and 1	POS
#6A	F/6	AMX/CL	MPE	Unknown [5°]	NEG	NEG	NEG	MPE + UA [2°]	2	POS
#7A	M/1	AMX/CL	ER	few [5°]	NP	NEG	NEG	U [1°]	1.20	NEG
#8A	M/3	AMX/CL	U U	Unknown [2°] 1.5 [4°]	NEG	NEG	NEG	UA[5°]	2	NEG
#9A	F/1	AMX/CL	U	0.10 [3°]	NP	NEG	NEG	MPE [4°]	Few	NEG
#10A	F/4	AMX	ER	1 [5°]	NP	NEG	NEG	MPE [4°]	Few	NEG
#11A	F/4	AMX	MPE	24 [10°]	NP	NEG	NEG	U [5°]	24	POS
#12A	F/6	AMX and AMX/CL	ED	few [1°]	NP	NEG	NEG	ED [3°]	2	NEG
#13A	F/11	AMX/CL	UA	7-8 [5°]	NP	NEG	NEG	U [3°]	>1	NEG
#14A	F/3	AMX/CL	U	0.30 [4°]	NP	NEG	NEG	U [5°]	12	NEG
#15A	F/3	AMX/CL	U	Unknown [7°]	NP	NEG	NEG	MPE [3°]	24	POS
#16A	M/4	AMX/CL	U	48 [5°]	NP	NEG	NEG	UA [7°]	48	NEG
#17A	M/2	AMX/CL	UA	few [1°]	NP	NEG	POS (3/3 after 20 min) (10/10 after 24 hrs)	ER [1°] and MPE [2°]	8 and 24	POS
#18A	M/3	AMX/CL	ER U	Unknown [1°] 8 [7°]	NP	NEG	NEG	MPE [2°]	7	NEG
#19A	M/6	AMX/CL	ER	Unknown [1°]	NP	NEG	NEG	ER [2°]	4	NEG
#20A	M/3	AMX	MPE	Unknown [2°]	NP	NEG	NEG	U [2°]	5	POS
#21A	F/10	AMX/CL	UA	few [5°]	NP	NEG	NEG	U [5°]	24	POS
#22A	M/7	AMX/CL	U + ED	10 [5°]	NP	NEG	NEG	U [4°]	Unknown	POS
#23A	F/6	Cefpodoxime proxetil	U	few [1°]	NP	NEG	NEG	U [6°]	>1	POS
#24A	M/1.9	AMX/CL	MPE	>1 [9°]	NP	NEG	NP	U [1°]	5	POS
#25A	F/5.3	AMX/CL	U	>1 [5°]	NP	NEG	NEG	MPE [5°]	12	POS

Note: Demographic characteristics, clinical pictures of the reaction due to the culprit, type of responsible drug, time interval between the drug intake and the reaction, results from the in vivo ed in vitro tests are detailed for the 25 children with positive drug provocation test (DPT) (group A). LTT was considered as positive when stimulation index (SI) was ≥3 in response to at least one molar concentration of amoxicillin or amoxicillin/clavulanic acid as specified into Appendix.[S9]

Abbreviations: AMX, amoxicillin; AMX/CL amoxicillin/clavulanic acid; ED, exfoliative dermatitis; ER, erythematous rash; F, fever; GI, gastrointestinal symptoms (vomiting and/or diarrhea); MPE maculopapular exanthema; NP, not performed; U, urticaria; UA, urticaria and angioedema.

TABLE 2 Clinical characteristics, in vivo and in vitro tests in DPT negative children

			Clinical history					
Pt. n°	Sex/age (yrs)	Culprit drug	Symptoms	Latency (hrs) [day of treatment]	PTs	SPT (wheal mm)	IDTs (wheal mm/erythema mm)	LTT
#1B	M/2	AMX/CL	MPE	few [3°]	NP	NEG	NEG	NEG
#2B	F/0.8	AMX/CL	MPE	3 [Unknown]	NP	NEG	NEG	NEG
#3B	F/12	AMX/CL	U	Unknown [3°]	NP	NEG	NEG	NEG
#4B	M/8	AMX	U	2 [unknown]	NP	NEG	NEG	NEG
#5B	M/7	AMX/CL	U	2 [2°]	NP	NEG	NEG	NEG
#6B	M/3	AMX/CL	U	Unknown [7°]	NP	NEG	NEG	POS
#7B	M/5	AMX/CL	U	few [3°]	NP	NEG	NEG	NEG
#8B	M/7	AMX/CL	MPE	8 [7°]	NP	NEG	NEG	NEG
#9B	M/6	AMX/CL	U	3 [1°]	NP	NEG	NEG	NEG
#10B	M/14	AMX/CL	MPE	8 [3°]	NP	NEG	NEG	NEG
#11B	F/2	AMX/CL	MPE	6 [1°]	NP	NEG	NEG	NEG
#12B	F/3	AMX/CL	U	Unknown [7°]	NP	NEG	NEG	POS
#13B	F/5	AMX/CL	U	few [5°]	NP	NEG	NEG	NEG
#14B	M/2	AMX/CL	MPE	8 [unknown]	NP	NEG	NEG	NEG
#15B	F/3	AMX/CL	ER	Unknown	NP	NEG	NEG	NEG
#16B	M/6	AMX/CL	U	12 [1°]	NP	NEG	NEG	NEG
#17B	M/5	AMX/CL	U	>1[7°]	NP	NEG	NEG	NEG
#18B	M/0.11	AMX/CL	U	4 [4°]	NP	NEG	NEG	NEG
#19B	M/1	AMX/CL	MPE	24 [7°]	NP	NEG	NEG	NEG
#20B	F/10	AMX/CL	U	6 [1°]	NP	NEG	NEG	NEG
#21B	M/2	AMX/CL	GI	>1 [1°]	NP	NEG	NEG	NEG
#22B	F/5	AMX/CL	U	8 [2°]	NP	NEG	NEG	NEG
#23B	F/16	AMX/CL	GI	Unknown [2°]	NP	NEG	NEG	NEG
			UA	Unknown [4°]				
#24B	M/4.5	AMX/CL	MPE	>1 [unknown]	NP	NEG	NP	NEG
#25B	F/5.7	AMX/CL	U	>1 [unknown]	NP	NEG	NEG	NEG

Note: Demographic characteristics, clinical pictures of the reaction due to the culprit, type of responsible drug, time interval between the drug intake and the reaction, results from the in vivo ed in vitro tests are detailed for the 25 children with negative drug provocation test (DPT) (group B). LTT was considered as positive when stimulation index (SI) was ≥3 in response to at least one molar concentration of amoxicillin or amoxicillin/clavulanic acid as specified into Appendix.[S9]

Abbreviations: AMX, amoxicillin; AMX/CL, amoxicillin/clavulanic acid; ED, exfoliative dermatitis; ER, erythematous rash; GI, gastrointestinal symptoms (vomiting and/or diarrhea); MPE, maculopapular exanthema; NP, not performed; U, urticaria; UA, urticaria and angioedema.

underwent cutaneous tests, and the day after started a 5-day DPT with the culprit.[S5] The timing and procedure of the work-up, in vivo and in vitro methods are all described in the Appendix.[S2-S8]

The 50 consecutively recruited children were divided into two groups: the group A was constituted by 25 children with positive DPT with the culprit (11 males; 14 females; range 1-14 years) whereas the group B included 25 children (15 males; 10 females; range 8 months-16 years) with negative DPT. All but one patient (#23A) reported reactions after AMX or AMX/CL, being AMX/CL prevalently involved (21/25 in group A and 24/25 in group B). Patient #23A was equally included despite a positive history after cefpodoxime proxetil as reacting with AMX/CL after DPT due to the possible cross-reactivity between penicillins and cephalosporins.[4] One patient (#12A) reported a double exposure (AMX first, then AMX/CL). The clinical characteristics of the patients are detailed in Tables 1 and 2 and summarized in Table[S1] in the Supporting Information. As expected, all the patients had negative SPTs. Only two patients, both in group A, underwent PTs with negative results. IDTs were performed in 49 with negative results in 48. The results of the in vivo tests are shown in Tables 1 and 2.

Lymphocyte transformation test was performed in all the patients with positive results in 13/25 in group A and 2/25 in group B (52% and 8%, respectively) (Tables 1 and 2, Table S2). In this way, when comparing the results coming from the two groups of patients, LTT showed a 52% sensitivity and 92% specificity with a 86% PPV and a 65% NPV. None of the children with negative history and tolerance to βLs exhibited LTT positivity (data not shown). In addition, when patients were grouped on the basis of their in vivo

(DPT + and -) and in vitro responses (LTT + and −), the difference between groups valuated by Fisher's exact test was highly statistically significant (*P* .0015) (Table S3). Cross-reactivity between AMX and AMX/CL was observed in 6/13 DPT+LTT+ patients and in both DPT-LTT+ children. Only one patient (#15A) was exclusively responsive to AMX/CL (Table S2).

Children commonly develop MPEs during viral infections but, for the frequent concomitant use of βLs, they are often inappropriately labelled as "penicillin allergic".[5] The diagnostic work-up is actually time-consuming, and a large number of painful IDTs is needed to often detect only a few true sensitizations. Thus, in mild delayed reactions, DPT is well accepted and performed directly without any previous skin test,[S2] as it is considered a safe procedure followed at most by mild reactions and is able to nicely identify sensitized patients.[6] However, how to perform it and how long to administer the culprit are still matters of debate.

The purpose of our study was to put together a complete in vivo and in vitro allergy work-up in a well-selected population of children who are homogenous in terms of age, sex, and type of responsible drugs (AMX or AMX/CL), numerically representative and susceptible to oral challenges with the culprit as a reliable method to define a true drug sensitization. In our hands, IDTs were unable to ascertain hypersensitivity as positive in a single patient (2%), which is in line with recently reported data in a larger population.[7] The diagnostic value of PTs was invaluable in our study as performed only in two patients, but the few available data in children with mild reactions to βLs, indeed show poor diagnostic sensitivity (1.7%).[5] The in vitro assay LTT, largely considered as a research tool, has been rarely investigated in children and was prevalently limited to severe reactions.[8] The novelty of the present work comes from the confident diagnosis of true or false βLs allergy based on provocation, which allows us to calculate sensitivity and specificity of in vitro lymphocyte proliferation. Our results are in agreement with previous data from adults, where the sensitivity of LTT ranges between 56% and 65% and specificity between 94% and 96%, with a PPV above 94%[9, S11, S12], whereas better performances have been reported in DRESS.[10]Thus, in children, DPT remains the gold standard in mild reactions given its high diagnostic capacity compared to IDTs. Although a negative result cannot exclude drug hypersensitivity, the good positive predictive value of LTT evaluated in our study may also support the diagnostic role of this in vitro assay also in those reactions to βLs where DPT cannot be performed.

KEYWORDS

amoxicillin, children, diagnostic accuracy, drug provocation test, lymphocyte transformation test

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Francesca Mori[1]
Lucia Fili[2]
Lucrezia Sarti[1]
Manuela Capone[2]
Giulia Liccioli[1]
Mattia Giovannini[1]
Simona Barni[1]
Elio M. Novembre[1]
Paola Parronchi[2]

[1]*Allergy Unit, Department of Pediatrics, Anna Meyer Children's University Hospital, Florence, Italy*
[2]*Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy*

Correspondence
Giulia Liccioli, Allergy Unit, Department of Pediatrics, Anna Meyer Children's University Hospital, Florence, Italy.
Email: giulialiccioli@gmail.com

ORCID

Giulia Liccioli https://orcid.org/0000-0002-5216-0423
Mattia Giovannini https://orcid.org/0000-0001-9568-6882
Paola Parronchi https://orcid.org/0000-0002-9184-5089

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SUPPORTING INFORMATION

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

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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