

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

To the Editor,

Beta-lactams (β Ls) 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 β Ls 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 β Ls, 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 β Ls 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 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 β Ls 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 β Ls, 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

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	Unknown [2°]	NEG	NEG	NEG	UA[5°]	2	NEG	
			U	1.5 [4°]							
#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	Unknown [1°]	NP	NEG	NEG	MPE [2°]	7	NEG	
			U	8 [7°]							
#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 and 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.⁵⁹

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

Pt. n°	Sex/age (yrs)	Culprit drug	Clinical history		PTs	SPT (wheal mm)	IDTs (wheal mm/erythema mm)	LTT
			Symptoms	Latency (hrs) [day of treatment]				
#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 and 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.⁵⁹

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.

often detect only a few true sensitizations. Thus, in mild delayed reactions, DPT is well accepted and performed directly without any previous skin test,⁵² 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 β Ls, 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 β Ls 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 β Ls 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.

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SUPPORTING INFORMATION

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

Groups	Sex (M/F)	Age range (years)	Culprit drug	Clinical history		DPT outcome		PTs	IDTs	LTT (%)
				Type of reaction†	Mean time latency (hrs from the last dose)	Type of reaction*	Mean time latency (hrs from last dose)			
A (DPT +)	11/14	1-14	• 21 AMX/CL • 3 AMX • 1 cefpodoxime proxetil	• 13 U • 5 MPE • 5 ER • 3 UA • 1 ED	• 9 • 8 unknown	• 12 U • 9 MPE • 3 ER • 3 UA • 1 ED	• 11,6 • 3 unknown	2 NEG 23 NP	1/24 POS 23/24 NEG 1 NP	13/25 POS (52)
B (DPT -)	15/10	0,8-16	• 24 AMX/CL • 1 AMX	• 14 U • 8 MPE • 1 UA • 1 ER • 2 GI	• 5,8 • 5 unknown	--	--	NP	24/25 NEG 1 NP	2/25 POS (8)
Controls	12/13	3-15	--	--	--	--	--	--	--	0/25 POS

Table S1. Summarized characteristics of the patients.

Patients were divided into two groups according to the result of drug provocation test (DPT): group A with positive DPT and group B with negative oral challenge. The additional group is represented by tolerant children without any history of reaction towards β -Lactams and used as control. AMX amoxicillin; AMX/CL amoxicillin/clavulanic acid

PTs patch tests, IDTs intradermal tests, LTT lymphocyte transformation test

ED exfoliative dermatitis, ER erythematous rash, GI gastrointestinal symptoms (vomiting and/or diarrhea), MPE maculopapular exanthema, U urticaria, UA urticaria and angioedema. NP not performed.

† Some patients showed more than 1 reaction

Pt. n°	LTT	mM concentration AMX (mg/ml)				mM concentration AMX/CL (mg/ml)				Pt. n°	LTT	mM concentration AMX (mg/ml)				mM concentration AMX/CL (mg/ml)			
		2,74 (1)	1,4 (0,5)	0,27 (0,1)	0,05 (0,02)	1,65 (0,5)	0,33 (0,1)	0,066 (0,02)	0,01 (0,004)			2,74 (1,0)	1,4 (0,5)	0,27 (0,1)	0,05 (0,02)	1,65 (0,5)	0,33 (0,1)	0,066 (0,02)	0,01 (0,004)
#1A	NEG	1,0	1,0	1,1	1,1	1,0	1,0	1,0	1,2	#1B	NEG	2,1	1,4	1,2	1,4	1,0	1,0	1,0	1,0
#2A	NEG	1,0	1,0	1,0	1,6	1,0	1,2	1,0	1,0	#2B	NEG	1,0	1,0	1,2	1,0	1,0	1,0	1,0	1,0
#3A	POS	5,5	5,6	5,0	1,7	0,6	4,0	5,4	2,6	#3B	NEG	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
#4A	POS	79,8	102,7	39,6	6,8	1,8	37,6	55,3	5,1	#4B	NEG	1,2	1,4	1,0	1,0	1,0	1,1	1,6	1,0
#5A	POS	6,9	13,2	7,5	11,4	1,6	9,4	7,4	10,4	#5B	NEG	1,0	2,0	1,3	1,5	1,0	2,8	1,4	1,5
#6A	POS	4,4	9,7	1,6	2,3	1,0	2,0	3,0	1,2	#6B	POS	7,0	3,3	2,8	1,0	1,0	4,0	2,1	1,3
#7A	NEG	1,0	1,3	1,2	1,7	1,0	2,0	1,0	1,1	#7B	NEG	1,6	1,6	1,0	1,3	1,0	1,0	1,0	1,1
#8A	NEG	1,0	1,1	1,0	1,0	1,0	1,0	1,0	1,1	#8B	NEG	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,1
#9A	NEG	2,2	2,3	1,0	1,0	1,0	1,2	1,0	1,4	#9B	NEG	1,0	1,0	1,0	1,0	1,0	1,3	1,0	1,0
#10A	NEG	1,1	1,0	1,0	1,0	1,0	1,0	1,0	1,0	#10B	NEG	1,0	1,1	1,0	1,0	1,0	1,2	1,0	1,3
#11A	POS	21,2	11,1	2,2	3,8	1,5	7,6	3,9	1,5	#11B	NEG	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
#12A	NEG	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	#12B	POS	5,7	7,5	1,5	1,0	1,0	7,1	1,2	1,0
#13A	NEG	1,0	1,0	1,0	1,3	1,0	1,0	1,0	1,0	#13B	NEG	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
#14A	NEG	2,9	2,7	1,1	1,8	1,0	2,1	2,1	2,3	#14B	NEG	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,1
#15A	POS	1,0	2,0	1,0	1,0	1,0	3,3	1,0	1,0	#15B	NEG	1,0	1,5	1,2	1,4	1,0	1,0	1,0	2,2
#16A	NEG	1,0	1,0	2,5	1,0	1,0	1,0	1,0	1,0	#16B	NEG	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
#17A	POS	7,4	1,9	1,1	1,0	1,3	2,9	1,0	1,0	#17B	NEG	1,7	1,0	1,0	1,0	1,0	1,0	1,0	1,0
#18A	NEG	1,4	1,1	1,0	1,0	1,0	1,0	1,0	1,0	#18B	NEG	2,0	1,0	1,1	1,2	1,0	1,0	1,0	1,0
#19A	NEG	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	#19B	NEG	1,2	1,0	1,0	1,0	1,0	1,0	1,0	1,0
#20A	POS	1,0	3,8	1,3	1,1	1,0	1,0	1,1	1,2	#20B	NEG	1,9	1,1	1,3	1,0	1,0	1,0	1,0	1,0
#21A	POS	4,7	3,2	1,5	1,4	1,0	2,3	1,2	1,0	#21B	NEG	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
#22A	POS	19,3	14,8	5,1	2,1	2,4	12,9	7,7	1,4	#22B	NEG	1,2	1,6	1,0	2,0	1,0	1,1	1,4	1,2
#23A	POS	3,2	1,2	1,0	1,7	1,0	1,8	2,7	1,0	#23B	NEG	0,4	0,8	0,9	1,1	0,4	0,4	0,3	1,1
#24A	POS	5,4	2,1	1,7	2,2	2,0	1,4	1,2	0,9	#24B	NEG	1,9	0,9	1,3	2,1	0,5	0,6	1,2	1,3
#25A	POS	2,0	2,6	1,8	6,0	1,4	1,6	2,7	1,8	#25B	NEG	2,1	1,0	1,1	1,1	0,8	1,4	1,1	1,1

Table S2. Lymphocyte transformation test in the paediatric population of the study

The stimulation indexes of the Lymphocyte Transformation Test are detailed for the 50 children with positive (group A) and negative (group B) drug provocation test. Positive results (SI $\geq$ 3,0) are reported in bold. AMX amoxicillin, AMX/CL amoxicillin/clavulanic acid.

	DPT +	DPT -	SUM
LTT +	13	2	15
LTT -	12	23	35
SUM	25	25	50

Table S3. 2x2 Contingency table.

DPT drug provocation test, LTT lymphocyte provocation test.

APPENDIX S1

SUPPORTING INFORMATION

Clinical history

Fifty children with a positive history of adverse drug reactions to amoxicillin (AMX) and/or amoxicillin/clavulanic acid (AMX/CL) were enrolled. The clinical history of the reaction was recorded at the time of the first allergic evaluation according to the European Network on Drug Allergy (ENDA) questionnaire.^{S1} The evaluation was programmed after at least a 4- to 6-week interval from the reaction. The clinical characteristics of the 50 patients are reported in Table S1. Twenty-five age- and sex-matched children tolerant to AMX or AMX/CL were additionally investigated as control. The parents of the studied patients or the control children group signed a written informed consent before testing. For all the tests, all the children had interrupted antihistamines or corticosteroids at least 10 days before the allergic work-up.

Cutaneous tests

Skin prick tests (SPTs) and intradermal tests (IDTs) with AMX/CL were carried out at the second evaluation in all the 50 patients. Mother solution and serial dilutions of the culprit drug were freshly prepared at the time of skin testing. IDTs were read after 20 minutes (immediate IDTs) and after 24–48 hours (delayed IDTs) according to ENDA.^{S2} Skin tests were considered as positive when the diameter was greater than 3 mm from the initial wheal or increased from the initial wheal in association with a flare and a negative saline control.^{S3} Patch tests (PTs) with AMX/CL at 5% and 20% in petrolatum with readings at 24–48–96 hours were performed in 2 out of 50 patients. A positive PT was defined whenever an infiltrate was detected.^{S4} All the 50 patients underwent a drug provocation test with the culprit drug the day after the completion of the skin tests.

Drug provocation test (DPT)

The oral challenge followed the protocol already described by Mori et al.^{S5} In brief, on day 1, AMX or AMX/CL were orally administered at refracted doses (1/10-2/10-7/10 of the pediatric therapeutic dose [25 mg/kg]) every 30 minutes in an inpatient setting. Patients were observed for 2 hours after the last drug intake in case of a negative outcome or up to 2 hours after the resolution of symptoms in case of reaction. If the first open challenge was negative, AMX or AMX/CL was administered the day after in one single dose. If DPT was negative, daily therapeutic doses of AMX or AMX/CL were prescribed at home for the following 5 days. Parents were thus advised to stop the treatment and to contact their child's primary care physician or hospital personnel in case of any kind of reaction. DPT was considered as positive if, during the challenge or within 48 hours after the end of the challenge, any objective skin, respiratory, cardiovascular, neurologic, or gastrointestinal symptom was observed and documented with a picture by a physician or by parents. If there was a reaction, the child was evaluated again in the Allergy Unit. The negative challenges were communicated by phone.^{S6}

Reagents

The culture medium was VLE-RPMI 1640 (Biochrom GmbH) supplemented with 2 mm endotoxin-free L-glutamine, 1% nonessential amino acids, 1% sodium pyruvate (Sigma-Aldrich), and 2×10^{-5} M 2-ME (Merck) (complete medium). AMX were purchased from Sigma; AMX/CL was purchased from GSK; rIL-2 (Proleukin) was purchased from Novartis; the polyclonal activator phytohemagglutinin (PHA) was purchased from Biochrom AG; and the recall antigen streptokinase (SK) was purchased from CSL Behring.

Lymphocyte transformation test

Peripheral blood mononuclear cells (PBMCs) were isolated by Lymphoprep (Sentinel Diagnostic). 2×10^5 cells were cultured in triplicate in round-bottomed 96-well plates in a complete medium, plus a 5% autologous serum, in a final volume of 0.2 mL for five days in the presence of four increasing

concentrations of individual β Ls (AMX 2.74-1.4-0.27-0.05 mM and AMX/CL 1.65-0.33-0.066-0.01 mM, corresponding to 1-0.5-0.1-0.02 and 0.5-0.1-0.02-0.004 mg/mL, respectively) or in a medium alone as a negative control at 37°C in 5% CO₂ humidified atmosphere. As positive controls, 2,500 UI/mL SK and 1% vol/vol PHA were used.^{S7} After 16-hour pulsing with 0.5 μ Ci/well ³HTdR (PerkinElmer), the cultures were harvested, and radionuclide uptake was measured by scintillation counting. A stimulation index (SI) (ratio between the radioactivity from stimulated and unstimulated cultures) ≥ 3 was considered as positive^{S8} and LTT was considered positive in case of positive proliferation against at least one of the concentrations of tested β Ls.^{S9}

LTT was performed not before 4 to 6 weeks from the reaction and within two years without the interference of corticosteroids.

Statistical Analysis

Sensitivity, specificity, and negative and positive predictive values were calculated using descriptive statistics.^{S10}

References

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