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TITLE PAGE

Letter to the Editor

Assessment of drug causality in SJS/TEN: concordance between lymphocyte transformation test and ALDEN

Short title: LTT and ALDEN: An analysis of concordance

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To the Editor,

Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) is the most severe drug-induced cutaneous adverse reaction (SCAR).¹ The high mortality and disabling sequelae highlights its clinical relevance.¹⁻³

SCARs are T cell-mediated hypersensitivity reactions.³ An eliciting drug can be identified in approximately 80% of SJS/TEN cases.⁴ Allopurinol, aromatic antiepileptics, antibacterial sulfonamides and sulfasalazine are frequently associated; although a great variety of medications might be involved.⁵ Prompt withdrawal of the culprit drug is the core of treatment in SJS/TEN.¹ However, its identification is often difficult, especially in polymedicated subjects.³ The algorithm of drug causality for epidermal necrolysis (ALDEN) is a 6-item scoring system validated in community-acquired cases, which translates the total score assigned to each drug into a probability category (<0: very unlikely; 0-1: unlikely; 2-3: possible; 4-5: probable and ≥ 6 : very probable).⁶ *In vivo* and *in vitro* diagnostic tests can also be used to identify the culprit drugs. As rechallenge is not acceptable, cutaneous tests are the only available *in vivo* tools. However, their sensitivity in SJS/TEN is low.³ Among *in vitro* tests, the lymphocyte transformation test (LTT), a lymphocyte proliferation assay, has been the most widely used in type IV hypersensitivity reactions.⁷ Nonetheless, its diagnostic value and its usefulness in SJS/TEN remain controversial.³

To evaluate the agreement between LTT and ALDEN, and to estimate the sensitivity and specificity of LTT in SJS/TEN using ALDEN as the standard in the acute phase and after recovery, we enrolled 30 patients within the SJS/TEN spectrum (Table S1). The study was approved by the Research Ethics Committee of Principe de Asturias University Hospital. Written informed consent was obtained for all patients. The phenotypes were clinically validated by an expert committee according to current classifications,² and blinded to drug exposures. LTT assays were performed as previously described.⁷ At least 4 concentrations were tested for each drug (Supplementary methods, and Table S2). As the standard criteria, a stimulation index (SI) ≥ 2 in at least one drug concentration tested was considered a positive result, with the exceptions of beta-lactam antibiotics (SI ≥ 3).⁷

Drug causality assessment was independently performed using ALDEN (Table S3) in each of 66 drugs tested by LTT in the 30 patients included in the study. Drugs newly introduced during the 8 weeks before and continued until the index day, or at least until 8 days prior to the index day, were investigated. Among them, 27 drugs scored as probable or very probable (ALDEN ≥ 4), 11 as possible (ALDEN 2-3) and 28 as unlikely or very unlikely (ALDEN ≤ 1). (Figure 1A and Table S4). We considered drugs scoring ≥ 4 as related to the reaction while drugs that scored < 4 were considered unrelated. Among the 30 patients studied, 23 (77 %) were exposed to at least one drug with a very probable or probable categories, 2 (7%) to drugs

with a possible category, and in 5 cases (16%) all drugs analyzed scored as unlikely or very unlikely (Figure 1B).

Fifty-six drugs were tested by LTT after recovery (Fig. S1), 22 related (ALDEN $\geq$ 4) and 34 unrelated (ALDEN <4). Eighteen drugs were tested during acute SJS/TEN, 6 related, and 12 unrelated. Individual results are shown in Table S4. Significantly higher ALDEN scores were found for drugs with positive LTT results after recovery (Figure 1C), while no significant differences were found in samples from the acute phase (Figure 1D). Interestingly, none of the drugs with only possible ALDEN scores (2-3) tested positive (Figure 1E,F). LTT was positive in 19/22 tests performed after recovery with related drugs, while it was negative in 25/34 unrelated drugs (sensitivity: 86.3%; specificity: 73.5%; $p<.0001$) (Table S5). The kappa statistic for agreement between LTT and ALDEN was .571, being the results similar when only community acquired cases were analyzed (Table 1). In order to explore the impact of rarely tested drugs, an analysis was performed considering only LTT results to drugs for which reliable test conditions had been published (Table S2), showing similar data (Table 1). LTT results were positive after recovery in 13/14 tests (92.85%) performed with high risk drugs (ALDEN notoriety 3). All of them scored as related (ALDEN $\geq$ 4), precluding the analysis using contingency tables. Interestingly, 10/18 low risk drugs (notoriety 1) were positive in LTT, 6 of them related (Figure S2). A ROC curve analysis including all drugs suggests a good diagnostic capacity of LTT to discriminate culprit from non-culprit drugs after recovery in SJS/TEN (AUC=.808) (Figure S3). The discrepancies with previous reports showing low sensitivity^{8,9} may rely on technical aspects, such as incubation periods or drug concentrations tested.

In the acute phase, 2/6 related drugs and 5/12 unrelated drugs tested positive, yielding to sensitivity and specificity values of 33% and 58.3%, respectively (Table S6). The result slightly improved when only community cases or selected drugs were analyzed; although no statistical significance was achieved (Table 1).

Our study has limitations, such as the low sample size regarding single drugs and acute phase analysis, and the use of ALDEN as the gold standard for causal relationship, which may have some weakness, such as protopathic bias for certain drugs, and the strong weight of the notoriety item⁶. However, the sensitivity and specificity of the LTT was similar when only drugs with moderate to high (notoriety 2-3) or low risk (notoriety $\leq$ 1) were examined (Table 1), suggesting that a positive LTT after recovery can be helpful to confirm the causal relationship suggested by ALDEN for drugs with low notoriety. Nonetheless, it is important to emphasize that a negative result does not exclude drug causality, especially for drugs with high ALDEN scores.

Our results suggest that LTT can be helpful for drug causality evaluation after recovery in patients who developed SJS/TEN after taking multiple medications. We agree with other authors that a combined

approach using other markers of cell activation could be useful to identify the causative agent. Clinical decisions regarding drug withdrawal still have to be taken on the basis of clinician's experience, and further research is needed to standardize and evaluate available *in vitro* tests in acute and resolution stages of the disease.³

REFERENCES

1. Paulmann M, Mockenhaupt M. Severe drug-induced skin reactions: clinical features, diagnosis, etiology, and therapy. *J Dtsch Dermatol Ges* 2015;13(7): 625-645.
2. Sekula P, Dunant A, Mockenhaupt M, et al. Comprehensive survival analysis of a cohort of patients with Stevens-Johnson syndrome and toxic epidermal necrolysis. *J Invest Dermatol* 2013;133(5):1197-1204.
3. Arden-Jones MR, Mockenhaupt M. Making a diagnosis in severe cutaneous drug hypersensitivity reactions. *Curr Opin Allergy Clin Immunol* 2019;19(4):283-293.
4. Bachot N, Roujeau JC. Differential diagnosis of severe cutaneous drug eruptions. *Am J Clin Dermatol* 2003;4(8):561-572.
5. Mockenhaupt M, Viboud C, Dunant A, et al. Stevens-Johnson syndrome and toxic epidermal necrolysis: assessment of medication risks with emphasis on recently marketed drugs. The EuroSCAR-study. *J Invest Dermatol* 2008;128(1):35-44.
6. Sassolas B, Haddad C, Mockenhaupt M, et al. ALDEN, an algorithm for assessment of drug causality in Stevens-Johnson Syndrome and toxic epidermal necrolysis: comparison with case-control analysis. *Clin Pharmacol Ther* 2010;88(1):60-68.
7. Pichler WJ, Tilch J. The lymphocyte transformation test in the diagnosis of drug hypersensitivity. *Allergy* 2004;59(8):809-820.
8. Kano Y, Hirahara K, Mitsuyama Y, et al. Utility of the lymphocyte transformation test in the diagnosis of drug sensitivity: dependence on its timing and the type of drug eruption. *Allergy* 2007;62(12):1439-1444.

9. Tang YH, Mockenhaupt M, Henry A, et al. Poor relevance of a lymphocyte proliferation assay in lamotrigine-induced Stevens-Johnson syndrome or toxic epidermal necrolysis. Clin Exp Allergy 2012;42(2):248-254.

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Author contributions

TB performed and analyzed the LTT assays.

SR, ER and FJdA performed the ALDEN analysis for all drugs tested.

RC, CGH, OG, ES, VL, AF selected and recruited the patients for the study.

TB, RC, OG, CGH, ES, VL, AM reviewed the cases for clinical validation.

RC, ER, SM, VL, FdA provided suggestions and discussions throughout the study

TB wrote the manuscript together with SR, FdA, VL, ER and RC

Table 1. Summary of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of LTT[†] in SJS/TEN drug causality assessment under different conditions and criteria.

Analysis performed after recovery	Sensitivity	Specificity	PPV	NPV	Agreement with ALDEN [‡] (Kappa Cohen's statistic)	Fisher's exact test
All drugs (N=56)	86.4 %	73.5 %	67.9 %	89.3 %	$\kappa=0.571$	P<.0001
Community cases (N=44 drugs)	88.2 %	77.8 %	71.4 %	91.3 %	$\kappa=0.633$	P<.0001
Selected drugs [§] with previously defined test conditions (N= 22 drugs)	92.9 %	62.5 %	81.3 %	83.3 %	$\kappa=0.585$	P=0.001
Drugs with notoriety 2-3 (N=22 drugs)	86.7 %	71.4 %	86.7 %	71.4 %	$\kappa=0.581$	P=0.014
Drugs with notoriety ≤1 (N=34 drugs)	85.7 %	74.1 %	46.2 %	95.2 %	$\kappa=0.454$	P=.007
Analysis performed in the acute phase	Sensitivity	Specificity	PPV	NPV	Agreement with ALDEN [§] (Kappa Cohen's statistic)	Fisher's exact test
All drugs (N=18)	33.0 %	58.3 %	28.6 %	63.6 %	$\kappa=0.08$	NS
Community cases (N=16 drugs)	50.0 %	58.3 %	28.6 %	77.7 %	$\kappa=0.06$	NS
Selected drugs [†] with previously defined	40.0 %	66.7 %	66.7 %	40 %	$\kappa=0.059$	NS

test conditions

(N= 8 drugs)

Drugs with notoriety

2-3	33.0 %	50.0 %	66.7 %	20 %	$\kappa = -0.111$	NS
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(N=8 drugs)

[†]LTT positive if SI ≥ 2 except for beta-lactams (SI ≥ 3) in at least one concentration tested.

[‡]ALDEN score ≥ 4 was considered as the reference for correct diagnosis.

NS: not significant

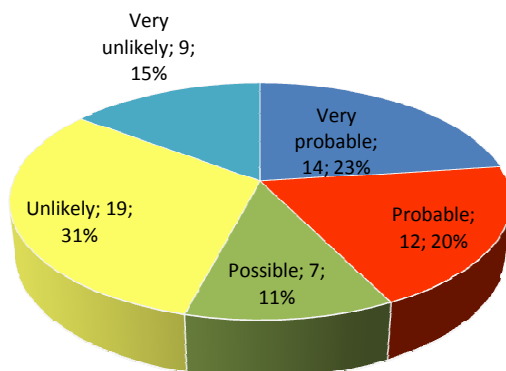
[§]Includes aromatic anticonvulsants, oxypurinol, sulfamethoxazol, sulfasalazine, ciprofloxacin, amoxicillin, penicillin V, and ceftriaxone

FIGURE LEGENDS

Figure 1. (A) ALDEN results for 66 drugs tested. Number and frequency of drugs in each category are shown. **(B)** Drug causality results in 30 SJS/TEN patients. The drug with the highest score was considered for each patient. The number and frequencies of patients within each category are shown. **(C).** LTT results in the recovery phase. Significantly higher ALDEN scores were obtained for drugs with positive LTT (Mann-Whitney U test) **(D)** LTT results in the acute phase. No significant difference in ALDEN scores was observed between positive and negative LTT. **(E)** Distribution of LTT results after recovery according to ALDEN scores. **(F)** Distribution of LTT results in the acute phase according to ALDEN scores.

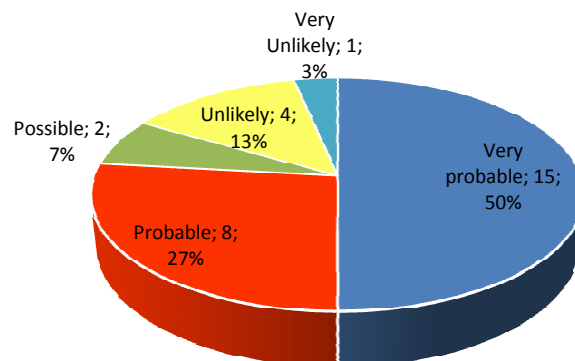
(A)

Distribution of drug causality among the 61 drugs tested



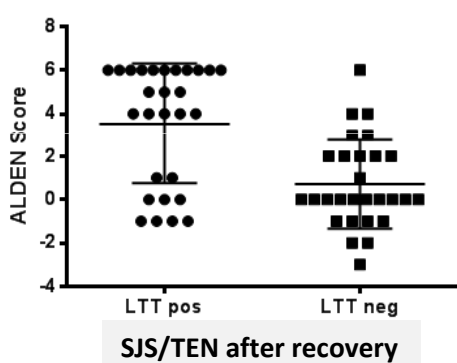
(B)

Distribution of drug causality among 30 SJS/TEN patients



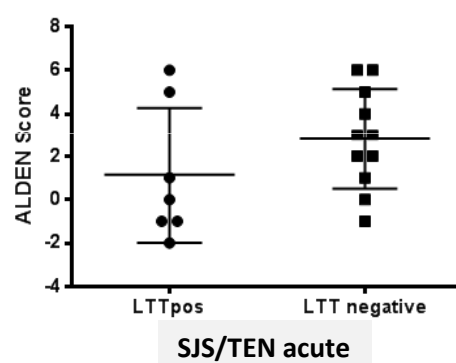
(C)

p=0.0003



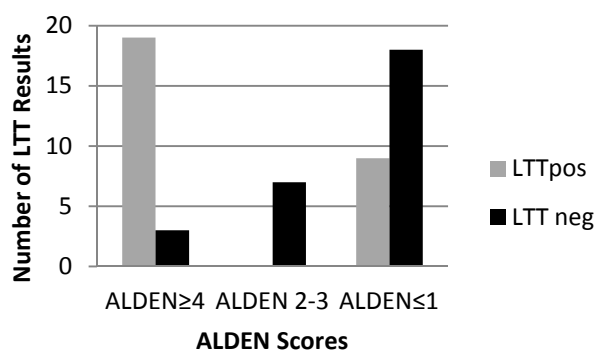
(D)

p=0.184



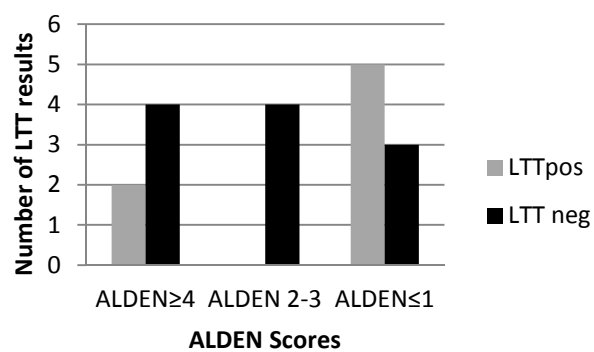
(E)

LTT after recovery of SJS/TEN



(F)

LTT in acute SJS/TEN



Supplemental methods

LTT assays

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation. Freshly isolated PBMCs were incubated during 6 days at 10^6 cells/mL in triplicates in round bottom 96 well plates and a final volume of 200 μ L. PBMCs were seeded in RPMI 1640 cell culture medium with 5% autologous serum and increasing concentrations of the suspected drugs. ^{3} H-thymidine (0.5 μ Ci/well) was added 18 hours prior to cell harvesting onto glass fiber filters, and ^{3} H incorporation to DNA was evaluated by scintillation counting. Unstimulated control cultures were established in the absence of drug, and positive control cultures were performed in the presence of 5 μ g/mL phytohemagglutinin (Sigma-Aldrich, St Louis, MO). A stimulation index (SI) was calculated as the ratio of ^{3} H incorporated by drug-stimulated cultures and by unstimulated cells.

As an exception to autologous serum, in one of the patients which was tested during the acute reaction and which had been treated with cyclophosphamide, 5% pooled human AB serum (Sigma-Aldrich) was used with a good response to the positive control.

Intravenous or parenteral pharmaceutical preparations were reconstituted and diluted in RPMI culture media. Pure substances were purchased from Sigma-Aldrich or provided by pharmaceutical companies. In some cases crushed pills were used.¹ Stock solutions were prepared in appropriate solvents before dilution in cell culture medium (Table S1). Preparation of drug solutions in RPMI 1640 was always done freshly just before use. Optimal drug concentrations were used according to available published data (see Table S2), or previous experience.^{2,3}

When no previous data were available, preliminary assays were performed using PBMCs from at least 2 healthy donors, starting with 1/500 dilutions of drug stock preparations. As a general rule, if a 1/500 dilution of the stock solution was exceeding more than 100-fold the maximum plasma levels reported, a 1/1000 dilution of the therapeutic dose was chosen as the starting concentration.

Some exceptions were dexamethasone, as positive responses had been described in type IV hypersensitivity reactions with glucocorticoid concentrations in the range of 1-10 μ g/mL,⁴ and vemurafenib, as functional analysis of antigen recognition showed preserved T cell function up to 10 μ M (5 μ g/mL).⁵ Nonetheless, higher concentrations (10 and 20 μ g/mL) were tested in 5 control donors showing toxic effects in lymphocytes after 6 days of culture.

Control cultures were established also in the presence of relevant solvent concentrations. Only non-toxic drug concentrations (SI >0.7 in PBMCs from healthy donors) were used for testing in PBMCs from patients. A range of at least 4 non-toxic concentrations was tested for each drug with the exceptions of patients from which a limited amount of sample was obtained, in which only 3 drug concentrations were tested.

Statistical analysis

The quantitative data are described as mean and standard deviation, or median, minimum, and maximum. The qualitative data are described as frequency and percentage.

The results of the LTT (positive/negative) were compared as a whole with the ALDEN results (related or unrelated drugs) using chi-squared test and Fisher's exact tests.

Sensitivity and specificity, positive predictive value (PPV), and negative predictive value (NPV) of LTT were calculated using 2x2 contingency tables using ALDEN as the gold standard as shown in the graph below

		DRUG CLASSIFICATION		
		Related (ALDEN≥4)	Unrelated (ALDEN<4)	Sum
LTT Result	Contingency Table			
	+	19 (TP)	9 (FP)	28
	-	3 (FN)	25 (TN)	28
	Sum	22	34	56

Parameter	Calculation	Measured
p-value	Fisher's exact test (two tailed)	<.0001
Sensitivity	$\frac{\text{True positives (TP)}}{\text{True positives (TP)} + \text{False negatives (FN)}}$	.8636
Specificity	$\frac{\text{True negatives (TN)}}{\text{True negatives (TN)} + \text{False positives (FP)}}$	.7352
PPV	$\frac{\text{True positives (TP)}}{\text{True positives (TP)} + \text{False positives (FP)}}$	.6785
NPV	$\frac{\text{True negatives (TN)}}{\text{True negatives (TN)} + \text{False positives (FP)}}$	.8928

The extent of agreement between the ALDEN algorithm and LTT results was analysed using the Cohen's kappa (κ) statistic.

Receiver operating characteristic (ROC) curves were built by a nonparametric method. The maximum SI obtained within the range of concentrations tested for each drug was considered for the analysis.

Stat pages (<http://statpages.info/ctab2x2.html>) and Graph Pad Prism 6 (La Jolla, CA, USA) were used to analyze the data.

Table S1. SJS/TEN Patients included in the study

Patient ID	PIELenRed /RegiSCAR	Community case	Sex	Age	Case Diagnostic	Case validation Score	Validation Committee	Nº drugs tested	Phase	Time from reaction
ID_1	5010001	Yes	F	34	SJS	Definite	RegiSCAR	3	Acute	8 d [†]
								3	Recovery	1.5 m [‡]
ID_2	5010005	Yes	M	82	SJS-TEN	Probable	RegiSCAR	3	Recovery	26 m
ID_3	5010014	No	M	48	SJS-TEN	Definite	RegiSCAR	1	Recovery	38 m
ID_4	5010035	Yes	M	3	SJS	Possible	PIELenRed	1	Recovery	22 m
ID_5	5010041	Yes	F	86	SJS	Possible	RegiSCAR	2	Acute	1 d
ID_6	5010052	Yes	M	16	SJS	Probable	PIELenRed	1	Recovery	108 m
ID_7	5010053	Yes	F	25	TEN	Definite	RegiSCAR	1	Recovery	132 m
ID_8	5010054	Yes	F	27	TEN	Probable	PIELenRed	2	Recovery	106 m
ID_9	5010056	Yes	M	55	TEN	Probable	PIELenRed	1	Recovery	132 m
ID_10	5010059	Yes	F	54	SJS	Definite	RegiSCAR	2	Acute	1 d
								2	Recovery	21 m
ID_11	5010060	Yes	M	89	TEN	Definite	PIELenRed	1	Recovery	3.5 m
ID_12	5010072	Yes	M	10	TEN	Definite	PIELenRed	1	Recovery	90 m
ID_13	5010094	No	M	50	SJS	Probable	RegiSCAR	2	Acute	2 d
ID_14	5010095	Yes	M	68	TEN	Definite	RegiSCAR	1	Recovery	4 m
ID_15	5010096	Yes	M	41	TEN	Definite	RegiSCAR	1	Recovery	5 m
ID_16	5010111	No	M	43	TEN	Definite	RegiSCAR	4	Recovery	23 m
ID_17	5010115	Yes	M	50	SJS-TEN	Definite	RegiSCAR	2	Recovery	10 m
ID_18	5010126	Yes	M	35	SJS	Definite	RegiSCAR	2	Recovery	5 m
ID_19	5010130	Yes	F	68	SJS	Definite	PIELenRed	4	Recovery	23 m
ID_20	5010135	Yes	M	63	SJS-TEN	Probable	PIELenRed	3	Acute	6 d
ID_21	5010138	Yes	M	50	SJS-TEN	Definite	PIELenRed	3	Acute	9 d
ID_22	5010143	Yes	M	2	SJS-TEN	Probable	PIELenRed	2	Recovery	4 m
ID_23	5010153	No	F	45	SJS-TEN	Definite	RegiSCAR	4	Recovery	22 m
ID_24	5010196	Yes	F	22	SJS-TEN	Definite	RegiSCAR	7	Recovery	2.5 m
ID_25	5010201	Yes	F	45	SJS-TEN	Definite	RegiSCAR	1	Recovery	6 m
ID_26	5010202	Yes	F	5	SJS-TEN	Probable	PIELenRed	3	Acute	4 d
								3	Recovery	6 m
ID_27	5010213	Yes	F	38	TEN	Definite	PIELenRed	2	Recovery	7 m
ID_28	5010227	Yes	M	4	TEN	Definite	PIELenRed	2	Recovery	12 m
ID_29	5010270	No	M	53	TEN	Definite	PIELenRed	3	Recovery	14 m
ID_30	5010283	Yes	M	35	TEN	Definite	PIELenRed	1	Recovery	3 m

[†]d: days[‡]m: months

Table S2: Drugs used in LTT, solvents and maximal concentrations

Drug	Solvent	Highest concentration [#] in LTT assay	Plasma levels after therapeutic doses
Alprazolam	MetOH	5µg/mL	20-40 ng/mL
Amoxicillin	Intravenous	500 µg/mL ^{1,6}	17 µg/mL
Azitromycin	Intravenous	25 µg/mL	3.63 µg/mL
Carbamazepine	DMSO	50 µg/mL ^{7,8}	4-12 µg/mL
Cefotaxime	Intravenous	100 µg/mL	38.9-214.4 µg/mL
Ceftriaxone	Intravenous	100 µg/mL ^{9,10}	80-250 µg/mL
Chlorpheniramine	RPMI	25 µg/mL	1-21 ng /mL
Ciprofloxacin	Intravenous	50 µg/mL ¹¹	1.4 µg/mL
Deanxit [®] ‡	RPMI	0.1 µg/mL [§]	1.7 ng/mL
Dexamethasone	intravenous	10 µg/mL ^{4*}	50-70 ng/mL
Dexketoprofen	Intravenous	50 µg/mL	1.8-3.8 µg/mL
Dextromethorphan	MetOH	1 µg/mL	14 ng/mL
Folic acid (Vitamin B9)	Intravenous	20 µg/mL	2-20 ng/mL
Ibuprofen	Intravenous	50 µg/mL	34 µg/mL
Imipenem	Intravenous	200 µg/mL	12-20 µg/mL
Ketoconazole [‡]	DMSO	10 µg/mL	3.2-4.6 µg/mL
Lamotrigine	DMSO	50 µg/mL ¹²	3-13 µg/mL
Levetiracetam	Intravenous	100 µg/mL	5-40 µg/mL
Levonorgestrel	EtOH	0.1 µg/mL	14.1 ng/mL
Levofloxacin	Intravenous	100 µg/mL ¹³	2.8-5.2 µg/mL
Lorazepam	DMSO	25 µg/mL	11-16 ng/mL
MDMA	RPMI	50 µg/mL	125-230 ng/mL
Meropenem	Intravenous	200 µg/mL	35.6 µg/mL
Metamizole	Intravenous	50 µg/mL ^{14,15}	10 ng/mL??
Mirtazapine [‡]	DMSO	5 µg/mL	1.5-199.3 ng/mL
Olsetamivir	RPMI	50 µg/mL	36.9-150 ng/mL
Oxypurinol	1N NaOH	100 µg/mL ^{16,17}	15-23 µg/mL
Paracetamol (Acetaminophen)	Intravenous	50 µg/mL ^{7,14}	10-20 µg/mL
Penicillin V	Intravenous	1000 µg/mL ^{1,6,14}	9.92 µg/mL
Phenylephrine	RPMI	2 µg/mL	1.35-4.49 ng/mL
Phenytoin	Intravenous	100 µg/mL ^{7,14,18}	10-20 µg/mL
Sulfamethoxazole	Intravenous	100 µg/mL ¹⁹	50-60 µg/mL
Sulfasalazine [‡]	DMSO	200 µg/mL ¹⁹	40 µg/mL
Tietilpirazine [‡]	DMSO	0.3 µg/mL	52 ng/mL
Tramadol	Intravenous	25 µg/mL	293 ng/mL
Trimethoprim	Intravenous	100 µg/mL	1-6 µg/mL
Vancomycin	Intravenous	100 µg/mL ²⁰	15-20 µg/mL
Vemurafenib [‡]	DMSO	5 µg/mL ⁵	50 µg/mL
Vitamin C	Intravenous	50 µg/mL	7-37 µg/mL

[#] See supplementary methods.

[§]0.1 µg/mL Melitracen plus 5 ng/mL Flupentixol

*Concentrations published for prednisolone and hydrocortisone

‡Crushed pills were used

Table S3. Algorithm of drug causality for Epidermal Necrolysis (ALDEN) (Modified from ²¹)

1. Delay between drug intake and index date:	
1. From 5 to 28 days [§]	Suggestive (score +3)
2. From 29 to 56 days [§]	Compatible (score +2)
3. From 1 to 4 days [*]	Likely (score +1)
4. >56 days	Unlikely (score -1)
5. Drug started on or after the index day	Excluded (score -3)
[*] Suggestive (score +3) in case of previous reaction to the same drug	
[§] Likely (score +1) in case of previous reaction to the same drug	
2. Pharmacokinetics	
1. Drug continued up to index day or stopped at a time less than five times the half-life	Definite (score 0)
2. Drug stopped at a time more than five times the half-life; but liver or kidney disfunction or suspected drug interactions	Doubtful (score -1)
3. Drug stopped at a time more than five times the half-life	Excluded (score -3)
3. Prechallenge /rechallenge:	
1. SJS/TEN after use of the same drug	Positive for disease and drug (score +4)
2. SJS/TEN after use of similar drug or other reaction with the same drug	Positive for disease or drug (score +3)
3. Other reaction after use of similar drug	Positive unspecific (score +1)
4. No known previous exposure to the drug	Not done/unknown (score 0)
5. Exposure to the same drug without reaction before or after EN	Negative (score -2)
4. Dechallenge:	
1. Drug stopped (or unknown)	Neutral (score 0)
2. Drug continued without harm	Negative (score -2)
5. Type of drug (notoriety) [‡]:	
1. Drug with "high risk" according to previous case-control studies	Strongly associated (score +3)
2. Drug with definite but lower risk according to previous case-control studies	Associated (score +2)
3. Previous reports, ambiguous epidemiology results, Summary of Product Characteristics (SmPC)	Suspected (score +1)
4. No data available	Unknown (score 0)
5. No evidence of association found in previous studies	Not suspected (score -1)
[‡] A notoriety list based on epidemiology studies can be found at http://www.regiscar.org/Office_1.html . Notoriety of drugs used in this study is listed in Table S4	
6. Other cause	
1. If at least other drug with a superior score (≥4), subtract one point from the score of each other drug taken by the patient.	(score -1)
The total score regarding the probability is established according to the following five categories: Very Unlikely (<0); Unlikely ((0-1); Possible (2-3); Probable (4-5); Very Probable (≥6)	

Table S4. Notoriety, ALDEN scores and LTT results in the acute phase (A) or after recovery (R) for each drug tested

Patient	Drug	Notoriety	ALDEN	LTT		Patient	Drug	Notoriety	ALDEN	LTT	
				A ^{&}	R [†]					A ^{&}	R [†]
5010001	Lamotrigine	3	5	+	+	5010135	Phenytoin	3	5	—	
	Amoxicillin	2	0	+	—		Paracetamol	0	-1	—	
	Lorazepam	-1	-2	+	—		Dexamethasone	1	2	—	
5010005	Azithromycin	2	4		—	5010138	Phenytoin	3	6	—	
	Tramadol	1	3		—		Folic acid	0	2	—	
	Ibuprofen	1*	6		+		Dexamethasone	1	3	—	
5010014	Allopurinol [§]	3	6		+	5010143	Amoxicillin	2	3		—
5010035	Lamotrigine	3	6		+		Ibuprofen	1*	2		—
5010041	Sulfamethoxazole	3	6	+		5010153	Alprazolam	-1	1		+
	Trimetoprim	0	2	—			Mirtazapine	1*	0		+
5010052	Carbamazepine	3	5		+		Vancomycin	1*	-1		+
5010053	Phenytoin	3	6		+		Ciprofloxacin	2	2		—
	Carbamazepine	3	6		+	5010196	Vitamin C	0	0		—
	Amoxicillin	2	2		—		Chlorfeniramine	0	0		—
5010056	Phenytoin	3	5		+		Paracetamol	0	0		—
5010059	Amoxicillin	2	0	—	—		Dextromethorfan	0	0		—
	Dexketoprofen	1*	1	+	—		Phenylephrine	0	0		—
5010060	Metamizole	1	6		+		MDMA	0	0		+ [‡]
5010072	Lamotrigine	3	6		—		Levonorgestrel	0	-1		+ [‡]
5010094	Levofloxacin	2	4	—		5010201	Sulfasalazine	3	6		+
	Sulfamethoxazole	3	6	—		5010202	Paracetamol	0	-1	+	—
5010095	Vemurafenib	1*	4		+		Ibuprofen	1*	-1	+	+
5010096	Allopurinol [§]	3	4		+		Penicillin V	1	1	—	+
5010111	Meropenem	1	0		—	5010213	Ibuprofen	1	-3		—
	Imipenem	1	0		—		Amoxicillin	2	0		+
	Olsetamivir	1	4		+ [‡]	5010227	Lamotrigine	3	6		+
	Ketoconazole	0	2		—		Levetiracetam	1	-1		—
5010115	Paracetamol	0	-1		—	5010270	Cefotaxime	1	4		+
	Ibuprofen	1*	-1		—		Vancomycin	1*	4		+
5010126	Allopurinol [§]	3	6		+		Dexketoprofen	1*	4		—
	Melitracen/ Flupentixol	0	2		—	5010283	Carbamazepine	3	6		+
5010130	Phenytoin	3	6		+						
	Ceftriaxone	2	-1		+						
	Vancomycin	1*	-2		—						
	Tietilpirazine	0	0		—						

[&] A: LTT results in the acute phase (+: positive; — : negative); [†] R: LTT results after resolution of SJS/TEN (+: positive LTT ; — : negative LTT)

*Notoriety in RegiSCAR table is 0. We decided to consider the notoriety as 1 due to the risk warning in the summary of Product Characteristics (SmPC).

[§]Oxypurinol was tested in patients exposed to allopurinol

[‡]Positive in at least two independent tests

Table S5. Performance of LTT in SJS/TEN patients after recovery

	ALDEN $\geq$ 4 related	ALDEN<4 nonrelated	Sum	Predictive value
LTT positive	19	9	28	PPV= 67.9% (95% CI= .540 - .755)
LTT negative	3	25	28	NPV=89.3% (95% CI= .754 - .970)
Sum	22	34	56	
Sensitivity (Se) and Specificity (Sp)	Se= 86.4% 95% CI= .687 - .961	Sp= 73.5% 95% CI= .621 - .799		

PPV: Positive predictive value; NPV: Negative predictive value

P<.0001 (Fisher's exact test)

κ = 0.571

Table S6. Performance of LTT results in acute SJS/TEN

	ALDEN $\geq$ 4 related	ALDEN<4 nonrelated	Sum	Predictive value
LTT positive	2	5	7	PPV= 28.6% (95% CI= .055 - .605)
LTT negative	4	7	11	NPV=63.3% (95% CI= .490 - .840)
Sum	6	12	18	
Sensitivity (Se) and Specificity (Sp)	Se= 33% 95% CI= .064 - .706	Sp= 58.3% 95% CI= .449 - .770		

PPV: Positive predictive value ;NPV: Negative predictive value

Fisher's exact test: not significant

κ = 0.08

Supplemental references

1. Pichler WJ, Tilch J. The lymphocyte transformation test in the diagnosis of drug hypersensitivity. *Allergy* 2004; 59:809–20.
2. Cabañas R, Calderon O, Ramirez E, *et al.* Piperacillin-induced DRESS: distinguishing features observed in a clinical and allergy study of 8 patients. *J Investig Allergol Clin Immunol* 2014; 24: 425–30.
3. Cabañas R, Calderón O, Ramírez E, *et al.* Sensitivity and specificity of the lymphocyte transformation test in drug reaction with eosinophilia and systemic symptoms causality assessment. *Clin Exp Allergy* 2018; 48: 325–33.
4. Buettiker U, Keller M, Pichler WJ, *et al.* Oral prednisolone induced acute generalized exanthematous pustulosis due to corticosteroids of group A confirmed by epicutaneous testing and lymphocyte transformation tests. *Dermatology* 2006; 213: 40–3.
5. Comin-Anduix B, Chodon T, Sazegar H, *et al.* The Oncogenic BRAF Kinase Inhibitor PLX4032/RG7204 Does Not Affect the Viability or Function of Human Lymphocytes across a Wide Range of Concentrations. *Clin Cancer Res* 2010; 16: 6040–8.
6. Luque I, Leyva L, Torres MJ, *et al.* In vitro T-cell responses to beta-lactam drugs in immediate and nonimmediate allergic reactions. *Allergy* 2001; 56: 611–8.
7. Mauri-Hellweg D, Bettens F, Mauri D, *et al.* Activation of drug-specific CD4+ and CD8+ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepine. *J Immunol* 1995; 155: 462–72.
8. Wu Y, Sanderson JP, Farrell J, *et al.* Activation of T cells by carbamazepine and carbamazepine metabolites. *J Allergy Clin Immunol* 2006; 118: 233–41.
9. Yawalkar N, Reimers A, Hari Y, *et al.* Drug-induced linear IgA bullous dermatosis associated with ceftriaxone- and metronidazole-specific T cells. *Dermatology* 1999; 199: 25–30.
10. Yawalkar N, Hari Y, Frutig K, *et al.* T cells isolated from positive epicutaneous test reactions to amoxicillin and ceftriaxone are drug specific and cytotoxic. *J Invest Dermatol* 2000; 115: 647–52.
11. Schmid DA, Depta JPH, Pichler WJ. T cell-mediated hypersensitivity to quinolones: mechanisms and cross-reactivity. *Clin Exp Allergy* 2006; 36: 59–69.
12. Naisbitt DJ, Farrell J, Wong G, *et al.* Characterization of drug-specific T cells in lamotrigine hypersensitivity. *J Allergy Clin Immunol* 2003; 111: 1393–403.
13. Demir S, Gelincik A, Akdeniz N, *et al.* Usefulness of In Vivo and In Vitro Diagnostic Tests in the Diagnosis of Hypersensitivity Reactions to Quinolones and in the Evaluation of Cross-Reactivity.. *Allergy Asthma Immunol* 2017; 9:347–59.
14. Nyfeler B, Pichler WJ. The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. *Clin Exp Allergy* 1997; 27: 175–81.
15. Herdeg C, Hilt F, Büchtemann A, *et al.* Allergic cholestatic hepatitis and exanthema induced by metamizole: verification by lymphocyte transformation test. *Liver* 2002; 22: 507–13.
16. Yun J, Marcaida MJ, Eriksson KK, *et al.* Oxypurinol Directly and Immediately Activates the Drug-Specific T Cells via the Preferential Use of HLA-B*58:01. *J Immunol* 2014; 192: 2984–93.
17. Chung W-H, Pan R-Y, Chu M-T, *et al.* Oxypurinol-Specific T Cells Possess Preferential TCR Clonotypes and Express Granulysin in Allopurinol-Induced Severe Cutaneous Adverse Reactions. *J Invest Dermatol* 2015; 135: 2237–48.
18. Karami Z, Mesdaghi M, Karimzadeh P, *et al.* Evaluation of Lymphocyte Transformation Test Results in Patients with Delayed Hypersensitivity Reactions following the Use of Anticonvulsant Drugs. *Int Arch Allergy Immunol* 2016; 170: 158–62.
19. Zawodniak A, Lochmatter P, Beeler A, Pichler WJ. Cross-Reactivity in Drug Hypersensitivity Reactions to Sulfasalazine and Sulfamethoxazole. *Int Arch Allergy Immunol* 2010 ; 153: 152–6.
20. Mennicke M, Zawodniak A, Keller M, *et al.* Fulminant liver failure after vancomycin in a sulfasalazine-induced DRESS syndrome: fatal recurrence after liver transplantation. *Am J Transplant* 2009; 9: 2197.
21. Sassolas B, Haddad C, Mockenhaupt M, *et al.* ALDEN, an algorithm for assessment of drug causality in Stevens-Johnson Syndrome and toxic epidermal necrolysis: comparison with case-control analysis. *Clin Pharmacol Ther* 2010; 88: 60–8.

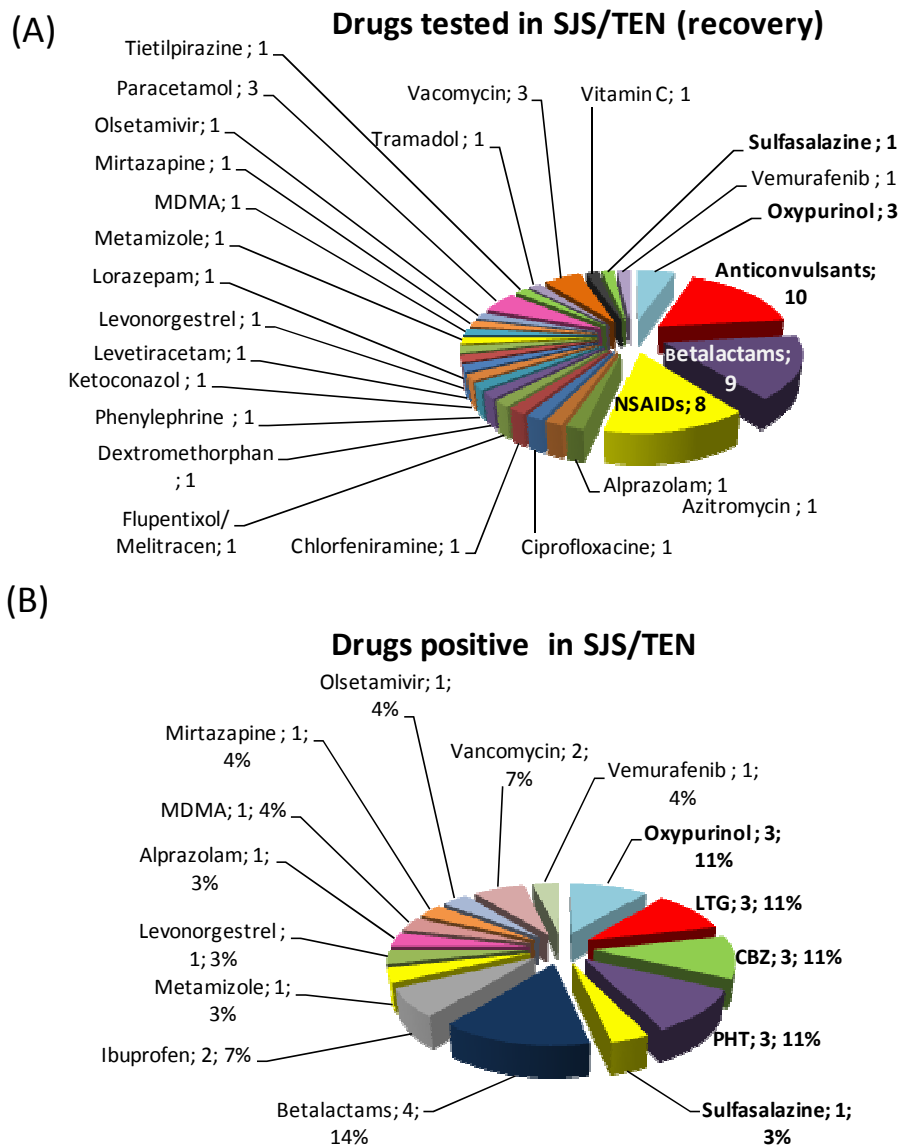
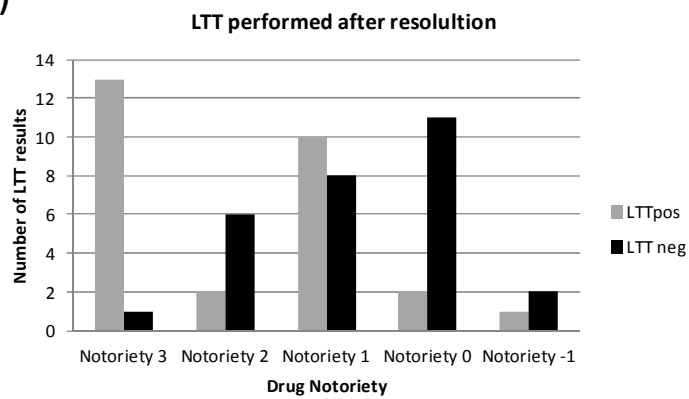


Figure S1. (A) Drugs used in the LTT assays analyzed in EN patients after recovery. **(B)** Drugs with positive LTT results in EN patients after recovery.

(A)



(B)

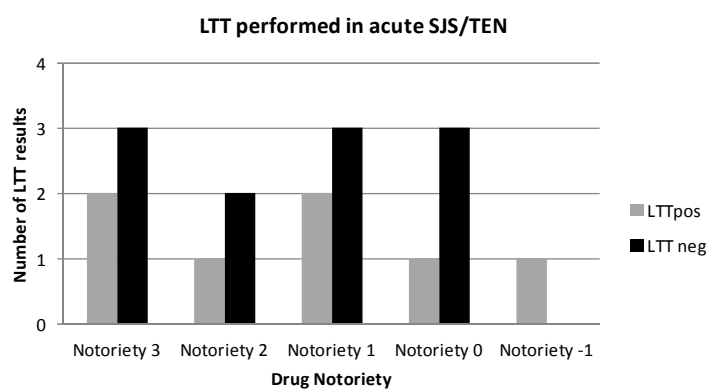
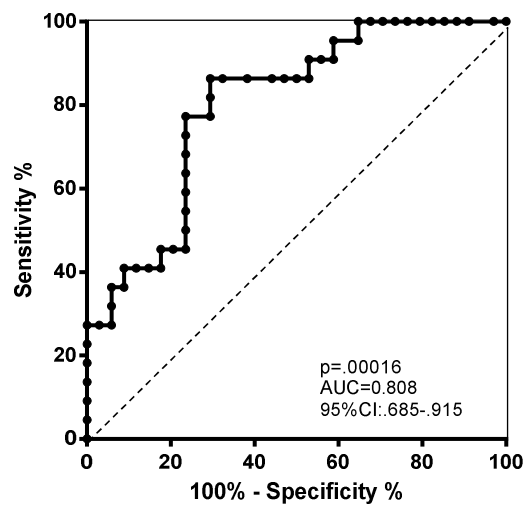


Figure S2. LTT results according to Drug Notoriety. LTT was performed in EN cases after recovery (A) or during the acute reaction or (B).

(A)



(B)

Phase	AUC (95% CI)	P-value	Cut-off (SI)	Sensitivity	Specificity
Recovery	.800 (.685-.915)	.00016	1.92	86.36%	67.50%
			2.02	86.36%	70.59%
			2.49	72.73%	76.47%
			2.96	54.55%	76.47%
			3.41	45.45%	82.35%

Figure S3. ROC curve analysis of LTT as culprit drug assessment tool in SJS/TEN. An ALDEN score $\geq$ 4 was used as standard for correct diagnosis. **(A)** ROC curve obtained with LTT results performed after recovery. **(B)** Summary of data obtained from the ROC curve analysis.