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Sensitivity and specificity of the lymphocyte transformation test in drug reaction with eosinophilia and systemic symptoms causality assessment

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Summary

Background: Drug reaction with eosinophilia and systemic symptoms (DRESS) is a severe delayed hypersensitivity reaction. The determination of drug causality is complex. The lymphocyte transformation test (LTT) has been reported positive in more than 50% of DRESS cases. Nevertheless, the sensitivity and specificity of LTT specifically in DRESS have not yet been established. Rechallenge with the culprit drug is contraindicated and cannot be used as gold standard for sensitivity and specificity determination.

Objective: To estimate the sensitivity and specificity of LTT in a clinically defined series of patients with DRESS.

Methods: Some 41 patients diagnosed with DRESS were included in the study. The results of the algorithm of the Spanish Pharmacovigilance System were used as the standard for a correct diagnosis of drug causality. A standard LTT was performed with involved drugs in acute or recovery samples. A stimulation index (SI) ≥2 in at least one concentration except for beta-lactams (SI ≥3) and contrast media (SI ≥4) was considered positive. Contingency tables and ROC curves were used for analysis.

Results: Sensitivity and specificity of LTT in the recovery phase of DRESS were 73% and 82%, respectively, whereas in the acute phase, they were only 40% and 30%, respectively. Comparison of skin tests and LTT confirmed a higher sensitivity and specificity of LTT in DRESS. LTT showed high sensitivity (S) and specificity (Sp) for anticonvulsants (S 100%, Sp 100%; $P = .008$), anti-TB drugs (S 87.5%, Sp 100%; $P = .004$), and beta-lactams (S 73%, Sp 100%; $P = .001$). ROC curves revealed that the best criteria for LTT positivity for all drugs are SI ≥2 in at least one concentration, increasing overall sensitivity to 80%, and for beta-lactams from 73% to 92%.

Conclusions and clinical relevance: LTT is a good diagnostic tool for drug causality in DRESS, mainly when performed in the recovery phase.

KEYWORDS
drug allergy, immunologic tests, T cells

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1 | INTRODUCTION

Drug reaction with eosinophilia and systemic symptoms (DRESS)[1] is a severe cutaneous adverse reaction (SCAR), also known as drug-induced hypersensitivity syndrome (DIHS).[2,3] DRESS/DIHS is a severe hypersensitivity type IVb drug reaction, with a mortality rate between 2 and 10 percent.[4-6] It is an entity of complex diagnosis because not all the signs and symptoms appear simultaneously. The score systems developed by the Japanese Research Committee on Severe Cutaneous Adverse Reaction (JSCAR)[7] and by the European Registry of Severe Cutaneous Adverse Reactions (RegiSCAR) group[8] are currently used for the diagnosis. DRESS is induced by drugs in more than 90% of cases.[6] Moreover, multiple drug sensitization, including sensitization to drugs introduced during the acute reaction, is not infrequent in DRESS cases.[9] The determination of drug causality is frequently a problem in severe reactions, given rechallenge is not feasible for ethical reasons[10] and recurrences can occur upon re-exposure.[11] Clinical judgement by experts is not always a reliable approach to determining the triggering agent in DRESS. Algorithms have been developed as an alternative tool to determine the causality likelihood of drugs taken by a given patient.[12] An allergy study, including skin tests (epicutaneous and intradermal tests) and in vitro tests, could also help to identify the etiological drug responsible for the reaction. In vitro tests have the advantage over in vivo diagnostic tests of being safe and are based on the property of antigen-specific T cells being activated upon stimulation with the nominal antigen in sensitized patients.[13] The lymphocyte transformation test (LTT) is the most widely used test, and it relies on the ability of drug-specific memory T cells to proliferate upon antigen stimulation. Various studies have aimed to establish the utility of LTT in the diagnosis of drug allergy[14-17]; however, there is controversy over the specificity and sensitivity of LTT in general. Significant variability has been reported in various series,[14-22] which could arise from the heterogeneity in the clinical entities and drugs tested. Some authors suggest that the LTT results might depend on the clinical entity and the timing of analysis.[23]

The lack of a gold standard precludes the accurate calculation of the sensitivity and specificity of LTT in SCARs, and no data exist on the sensitivity and specificity of LTT specifically in DRESS. Pichler and Tilch[15] found positive LTT results in more than 50% of DRESS cases. Nevertheless, the sensitivity and specificity of this test specifically in DRESS have not been established.

In this study, we aimed to estimate the sensitivity and specificity of LTT in DRESS. As an alternative to drug rechallenge as the gold standard, we have used the Algorithm of the Spanish Pharmacovigilance System (ASPS)[24] for drug causality assessment.

2 | PATIENTS AND METHODS

2.1 | Patients

An observational retrospective analysis was performed on collected clinical data from 41 patients diagnosed with DRESS syndrome from 2007 to 2013 at La Paz University Hospital. The study was approved by the local Ethics Committee (Code PI-1674) and was conducted in accordance with principles from the Declaration of Helsinki. All cases were reported to the Spanish Pharmacovigilance System. Some of the cases were included in the PIELenRED registry (http://pielenred.hol.es/PIELenRed/) and in the international registry RegiSCAR (www.regiscar.org) (Table S1). The study also included 35 additional patients with non-immediate drug-induced reactions in which a re-exposition drug test[25] was performed.

2.2 | Diagnosis of potential DRESS cases

The diagnosis of DRESS syndrome was established according to the diagnostic criteria proposed by RegiSCAR.[8] Patients with a score ≥2 (possible, probable, or definite cases) were included in the study.

2.3 | Allergological studies

The patients were studied at the allergy department of La Paz University Hospital (except 4 of the PIELenRed cases) after discharge. A detailed anamnesis and allergological workup were performed, including epicutaneous,[26,27] prick, and intradermal tests,[28] to identify the eliciting drug.

2.4 | LTT assays

LTTs were performed with every possible drug involved, as previously described.[15,29] Briefly, peripheral blood mononuclear cells were stimulated with increasing concentrations of the suspected drugs over 6 days in the presence of 5% autologous serum, and the proliferation was evaluated through the incorporation of ^{3}H-thymidine to DNA. Positive control cultures were performed in the presence of phytohaemagglutinin (Sigma). A stimulation index (SI) was calculated as the ratio of ^{3}H incorporated by drug-stimulated cultures and basal ^{3}H incorporation by unstimulated cells. As the standard criteria, SI ≥2 in at least one concentration was considered positive, with the exceptions of beta-lactam antibiotics (SI ≥3), and iodinated contrast media (SI ≥4), as previously suggested.[15] For comparative purposes, we analysed the data using 3 additional criteria: (i) LTT was considered positive using the SI standard criteria as above in at least 2 different concentrations; (ii) positive if SI ≥5 in at least one concentration; and (iii) positive if SI ≥5 in more than one concentration. Acute samples were obtained from patients during hospitalization and less than 2 weeks from the index date. LTT was performed during the acute phase of the reaction and/or after recovery (Table S1) at least 1 month after steroid treatment was stopped.

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, and stock solutions were prepared in appropriate solvents before dilution in culture medium (Table S2). In some cases, capsule contents or crushed pills were used.[15]

2.5 | Algorithm of the Spanish Pharmacovigilance System for drug causality assessment

Causality assessment was also performed using the ASPS.[24] This algorithm evaluates the following parameters: the chronology, the degree of knowledge of the relationship between the drug and the effect, evaluation of drug withdrawal, the rechallenge effect, and alternative causes. The final evaluation is listed as improbable (<0, not related), conditional (1-3, not related), possible (4-5, related), probable (6-7, related) or defined (>8, related) (Table S3).

All drugs taken during the exposure windows were recorded (including chronology of drug intake, dose, indication, and clinical course after drug withdrawal). A suggestive chronology was considered if the drug was initiated less than 6 months previously and stopped less than 14 days before the index day.[8] The index day was deemed to be the day on which prodromal symptoms/signs first occurred, or in its absence, the day of acute rash. The score for causality likelihood was evaluated separately by two clinical pharmacologists.

2.6 | Statistical analysis

The quantitative data are described as mean, standard deviation, median, minimum, and maximum. The qualitative data are described as frequency and percentage. The chi-square test and Fisher's exact tests were used to compare the results of the LTT (positive/negative) as a whole with the ASPS results (related or unrelated drugs as explained above). LTT results in independent therapeutic groups of drugs were analysed if at least n = 7.

Sensitivity and specificity, positive predictive value (PPV), and negative predictive value (NPV) of ASPS were calculated using 2×2 contingency tables. Positive re-exposition to the drug was used as the gold standard. A second analysis of sensitivity and specificity was made for LTT using ASPS as the standard criteria.

The extent of agreement between clinical pharmacologists and between the ASPS algorithm and re-exposition or LTT results were analysed using the κ statistic.

Receiver operating characteristic (ROC) curves were built by a nonparametric method. In this case, each individual result for every non-toxic drug concentration tested was considered for ROC curve analysis.

SPSS-15 software (IBM, Inc., Chicago, Il, USA) was used to analyse the data.

3 | RESULTS

A retrospective analysis was performed on collected clinical data from 41 patients diagnosed with DRESS syndrome from 2007 to 2013. A retrospective study was also performed including 35 additional patients with non-immediate drug-induced reactions in whom a re-exposition drug test[25] had been performed. Of 41 DRESS cases included in the study, 26 were women (63%) and 15 were men (37%). The median age was 61 years (range 7-89). Among them, 11 (27%) were definite DRESS, 14 (34%) were probable, and 16 (39%) were possible cases.[8] Drug causality in DRESS was assessed for a total of 273 drugs (median of 6 drugs, range 1-15); of these, 111 were concluded as being related drugs (ASPS score ≥4). The kappa index showed an almost perfect agreement between clinical pharmacologists (κ=.86) in the causality assessment. We performed 141 LTTs (111 in the recovery phase on 37 patients, and 30 LTTs on 12 cases during the acute phase). A median of 3 drugs was tested on each patient (range 1-8 drugs). Each drug was tested in a range of 4-5 non-toxic concentrations. In total, 57 drugs were tested (Figure 1 and Table S2).

Sixty-one skin tests (STs) were performed on 26 patients (48 with related drugs, and 13 with unrelated drugs according to ASPS). A median of 2 drugs was tested on each patient (range 1-5 drugs). Only 18 STs were positive (35.4% sensitivity and 92.3% specificity) (Table S4). Among the related drugs tested, 70% had positive LTT results, whereas only 35% had positive STs. Among the non-related drugs according to ASPS, only one had a positive ST, which was also clearly positive in the LTT (SI >5).

3.1 | Evaluation of the ASPS algorithm as a standard for drug causality assessment

To explore the suitability of the ASPS (related drugs, ASPS ≥4) as the standard for a correct diagnosis, the sensitivity and specificity of ASPS were initially calculated using the in vivo response to the drugs as the true gold standard. Although provocation tests are contraindicated in DRESS,[10] data were available from 62 drug re-expositions in 43 patients with various clinical entities: 8 patients with DRESS, 30 patients with exanthema/urticaria, 3 with acute generalized exanthematous pustulosis (AGEP), and 2 patients with Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). In all cases, the drugs were reintroduced either inadvertently or in patients with a negative drug allergy study, and in whom the drug was very important for the patient's therapeutic management. In this analysis, the sensitivity of ASPS was 100%, with 100% negative predicted value (NPV). Moreover, a very good degree of agreement between ASPS and the re-exposition data was obtained (κ=.723) (Table 1). These results support that ASPS is a suitable standard approach for evaluation of the diagnostic capacity of LTT assays.

3.2 | Sensitivity and specificity of LTT in DRESS

A total of 30 tests were performed on 12 patients during acute DRESS, less than 2 weeks after onset and before steroid treatment. According to the ASPS, 20 tests were performed with related drugs and 10 with non-related drugs. Individual results for each patient are shown in Figures S1 and S2.

Considering the standard SI cut-off for positivity [SI ≥2 in at least one concentration except for beta-lactams (SI ≥3), and iodinated contrast media (SI ≥4)], the sensitivity and specificity of LTT in acute

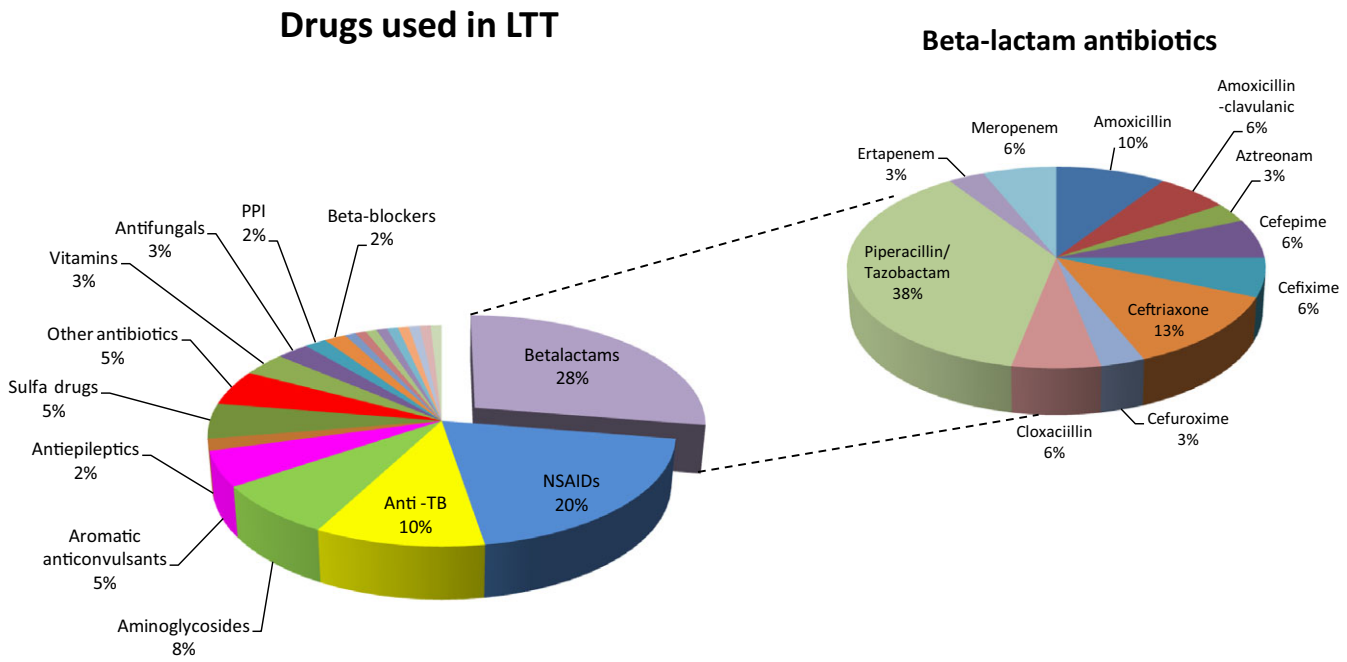


FIGURE 1 Drugs used in the LTT assays

TABLE 1 Contingency table: Specificity and sensitivity of ASPS in drug-induced non-immediate hypersensitivity reactions to medications using re-expositions as gold standard

	In vivo re-exposure		
ASPS	**Positive**	**Negative**	**Sum**
Related drugs (ASPS ≥ 4)	**18**	8	26
Non-related drugs (ASPS ≤ 3)	0	**36**	36
Sum	18	44	62[a]

P-value < .0001 (χ^2 test); *P*-value < .0001 (Fisher's exact test).
ASPS sensitivity = 100%; specificity = 81%; PPV = 69.2%; NPV = 100%; κ = .723; 95% CI: 0.495-0.723.
[a]Data from 43 patients with various drug-induced hypersensitivity skin reactions (DRESS; N = 8; Exanthema/urticaria: N = 30; AGEP: N = 3; SJS/TEN: N = 2).
Bold values represent concordant figures: related drugs with positive in vivo re-exposure and unrelated drugs with negative in vivo re-exposure test.

DRESS were 40% and 30%, respectively. We explored whether the results could be improved if more stringent conditions for positivity were applied (SI ≥2 or ≥5 in at least 2 drug concentrations tested). The sensitivity was decreased in these scenarios, although the specificity reached 90%; however, the results were not statistically significant (Figure 2A).

We performed 111 LTTs in the recovery phase of 37 DRESS cases, 28 tests with non-related drugs, and 83 with related drugs according to the ASPS. Considering the standard cut-off for positivity, the sensitivity and specificity of LTT in the recovery phase were 73.5% and 82.1%, respectively. No improvement was obtained when more stringent criteria were applied (Figure 2B).

The data indicate that LTT is a reliable tool for diagnosis of drug causality in the recovery phase of DRESS and that standard criteria for positivity are appropriate for a good balance between sensitivity and specificity.

Moreover, considering the in vivo re-exposition to the drug as the gold standard in the 7 DRESS cases in which drugs tested in the recovery phase were re-administered, LTT sensitivity in the recovery phase of DRESS was 100%, with 100% NPV (Table 2).

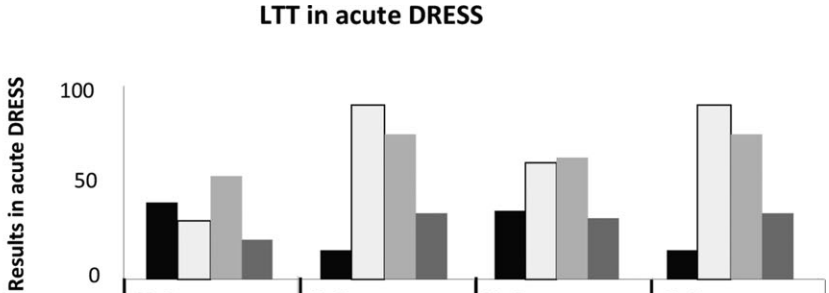
Comparisons between the results of LTT in the recovery phase and STs in the same patients are shown in Tables S5 and S6.

Because it has been suggested that the results of the test might depend on the drug involved,[14,30] LTT results were analysed according to the therapeutic group of drugs. A global analysis was also performed with those drugs more frequently involved in DRESS (aromatic anticonvulsants, allopurinol, and sulfamethoxazole).[4,5] Among them, none of the patients tested upon recovery had been exposed to allopurinol, and only a few cases had been tested with sulfamethoxazole. Therefore, we grouped anticonvulsants and sulfamethoxazole for analysis. A summary of the data is shown in Table 2. Although good specificity and PPV were obtained in the group of beta-lactam antibiotics, NPV in this group was low compared with other drug families analysed, especially with those families most frequently involved in DRESS.

3.3 | ROC curve analysis of the LTT as a diagnostic tool in DRESS

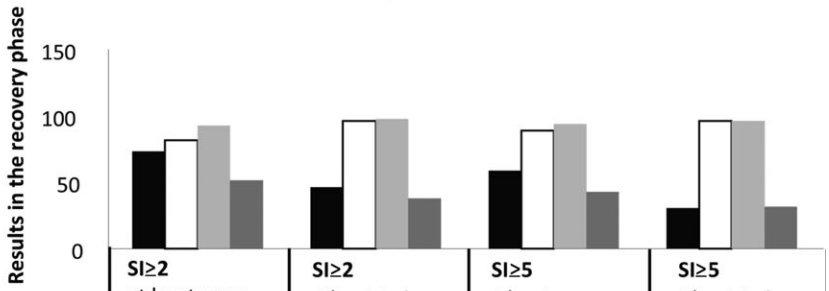
ROC curves were constructed to further evaluate the diagnostic capacity of LTT in DRESS and to estimate the optimal SI cut-off value. An ASPS score ≥4 was used as the standard for correct diagnosis, as above. LTT results obtained for all drugs in the acute or recovery phases of DRESS were analysed separately. A ROC curve was also constructed with LTT results from beta-lactam antibiotics in the recovery phase. In agreement with previous results, the ROC

(A)



	SI≥2 at least once			SI≥2 at least twice			SI≥5 at least once			SI≥5 at least twice		
	ASPS		Sum	ASPS		Sum	ASPS		Sum	ASPS		Sum
	R (≥4)	NR (≤3)		R (≥4)	NR (≤3)		R (≥4)	NR (≤3)		R (≥4)	NR (≤3)	
LTT positive	**8**	7	15	**3**	1	4	**7**	4	11	**3**	1	4
LTT negative	12	**3**	15	17	**9**	26	13	**6**	19	17	**9**	26
Sum	20	10	**30**	20	10	**30**	20	10	**30**	20	10	**30**
■ Sensitivity (%)	40			15			35			15		
□ Specificity (%)	30			90			60			90		
■ PPV (%)	53.3			75			63.6			75		
■ NPV (%)	20			34.6			31.6			34.6		
P-value*	NS			NS			NS			NS		

(B)



	SI≥2 at least once			SI≥2 at least twice			SI≥5 at least once			SI≥5 at least twice		
	ASPS		Sum	ASPS		Sum	ASPS		Sum	ASPS		Sum
	R (≥4)	NR (≤3)		R (≥4)	NR (≤3)		R (≥4)	NR (≤3)		R (≥4)	NR (≤3)	
LTT positive	**61**	5	66	**38**	1	39	**49**	3	52	**25**	1	26
LTT negative	22	**23**	45	45	**27**	72	34	**25**	79	58	**27**	85
Sum	83	28	**111**	83	28	**111**	83	28	**111**	83	28	**111**
■ Sensitivity (%)	73.5			45.8			59			30.1		
□ Specificity (%)	82.1			96.4			89.3			96.4		
■ PPV (%)	92.4			97.4			94.2			96.2		
■ NPV (%)	51.1			37.5			42.4			31.8		
P-value*	<.0001			<.0001			<.0001			.004		

FIGURE 2 Sensitivity, specificity, and positive and negative predictive values of LTT as a diagnostic tool in the acute stage (A) or after recovery of DRESS (B). Drugs were considered related if ASPS results were ≥4. Cases in which SI ≥2 was considered as positive, exceptions were established for beta-lactams (SI ≥3) and iodinated contrast media (SI ≥4). Contingency tables were built for the calculation of specificity and sensitivity. *Fisher's exact test results are shown (*P*-value). R: related drugs; NR: non-related drugs. NS: Not significant

curve suggests a poor diagnostic capacity of the test in the acute phase of DRESS (Figure 3A); however, an acceptable result (area under the curve [AUC]=.780) was obtained in the recovery phase. The results were even better when only beta-lactams were analysed (AUC=.890) (Figure 3B).

The Youden index was calculated to estimate the best cut-off for positivity. When all drugs were considered, an SI = 1.58 appeared to be the best cut-off, with a sensitivity of 64.97% and specificity of 78.85%. In the case of beta-lactams, the best point was obtained for an SI = 1.82, with 75.45% sensitivity and 100% specificity. For

TABLE 2 Summary of sensitivity and specificity of LTT during the recovery phase of DRESS according to various standards for correct diagnosis or to the drug therapeutic group

	N	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Fisher's exact test (*P*)
Gold standard						
ASPS ≥ 4	111	73.3	82.1	92.4	51.1	<.0001
Re-exposure	11	100	62.5	50	100	NS
Therapeutic group						
Anticonvulsants[a,b]	7	100	100	100	100	.008[c]
Anticonvulsants[b] + SMX[a]	12	100	100	100	100	.001[c]
Anti-TB[a]	12	87.5	100	100	80	.004[c]
Beta-lactams[a]	32	73.1	100	100	46.2	.001
NSAIDs[a]	13	77.8	66.7	77.8	66.7	NS
Metamizol[a]	9	100	25	57	100	NS
Vancomycin[a]	7	100	33	66.7	100	NS

N, Number of tests; NSAIDs, nonsteroidal anti-inflammatory drugs; SMX, sulfamethoxazole; NS, not significant.
LTT is positive if SI ≥ 2 except for beta-lactams (SI ≥ 3) or iodinated contrast (SI ≥ 4) in at least one concentration tested.
[a]ASPS ≥ 4 was considered as standard for correct diagnosis.
[b]Including one additional test with phenytoin in a patient not previously exposed.
[c]Pearson χ^2 test.

(A)

Drugs	Phase	AUC (95% CI)	*P*-value	Cut-off (SI)	Sensitivity	Specificity
All drugs	Acute	0.557 (0.487-0.687)	.101			
	Recovery	0.760 (0.710-0.811)	<.0001	**1.58**	64.97%	78.65 %
				1.99	52.14 %	86.52 %
				2.00	51.87 %	86.52 %
Betalactams	Recovery	0.890 (0.835-0.946)	<.0001	**1.82**	75.45 %	100 %
				2.00	72.00 %	100 %
				3.00	45.00 %	100 %

(B)

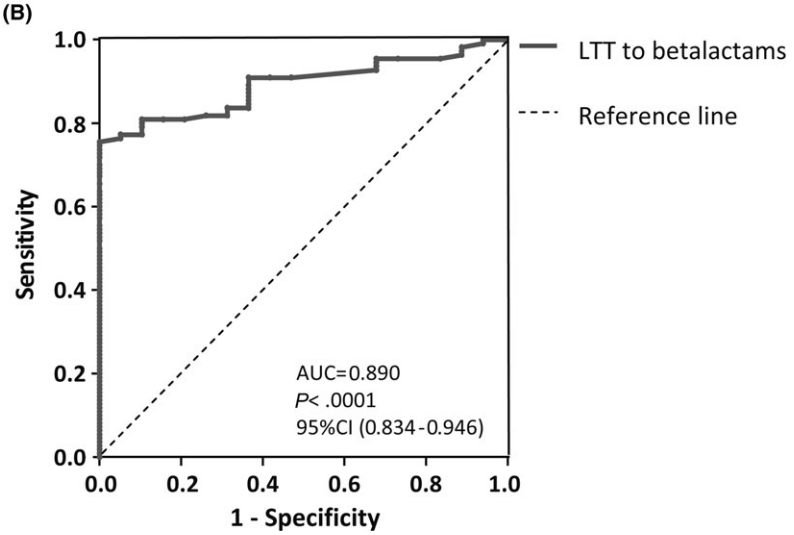


FIGURE 3 ROC curve analysis of LTT as a diagnostic tool in beta-lactam-induced DRESS, using ASPS ≥4 as standard for correct diagnosis. (A) Summary of data obtained from ROC curves built with results of LTT performed in acute or recovery phases of DRESS. The Youden index was calculated to estimate the SI cut-off points with best specificity and sensitivity; (B) ROC curve obtained with LTT results obtained with beta-lactam antibiotics in the recovery phase of patients with DRESS. SI, stimulation index; CI, confidence interval; AUC, area under the curve

stimulation indexes higher than 1.82, the false positive rate was 0 (1-specificity). However, considering SI = 3 as the cut-off point for positivity, the sensitivity of LTT to detect related drugs (here identified as those with ASPS score ≥4) was reduced considerably. On the other hand, an SI ≥2 as the cut-off point still showed good sensitivity (72%), with optimal specificity (100%) (Figure 3A).

3.4 | Sensitivity and specificity of the LTT in DRESS with homogeneous criteria for all drugs

In the light of the results obtained after ROC curve analysis, we again analysed beta-lactam LTT data considering an SI ≥2 positive (instead of 3, as classically recommended[15]). The sensitivity increased

from 73.1% to 92.3%, while specificity continued to be 100% (Fisher's exact test, $P < .001$). A substantial improvement in the NPV was also obtained.

The sensitivity and specificity of the LTT performed after resolution of DRESS were again calculated, considering SI ≥2 as positive for all drugs tested. The sensitivity increased from 74% to 80%. However, the best agreement between LTT results and ASPS for identification of the culprit drug was obtained in beta-lactam-induced DRESS (κ=.818) (Table 3).

4 | DISCUSSION

There is controversy over the specificity and sensitivity of LTT in general, with high variability reported in various series, which might depend on the heterogeneity in clinical entities, timing, drugs tested, and read-out systems.

Particularly in DRESS, most information is derived from small studies involving few patients or case reports suggesting its usefulness.[9,23,29,31-36] Pichler and Tilch[15] reported positive results in more than 50% of DRESS cases. Nevertheless, the sensitivity and specificity of LTT specifically in DRESS have not thus far been established. We report herein the largest DRESS series analysed using LTT and provide the first data on sensitivity and specificity of LTT in DRESS.

Various "gold standards" have been used to calculate the sensitivity and specificity of LTT. Nyfeler and Pichler[14] calculated the "probability of a drug allergy" based on the clinical history and provocation tests. Other authors used skin tests, the radioallergosorbent test, and/or controlled administration of the drugs as the reference methods.[16] A drug provocation test is the gold standard for the identification of the drug eliciting a hypersensitivity reaction[25]; nevertheless, it is unethical and contraindicated in severe reactions.[10] This difficulty hinders the validation of diagnostic tools for drug causality in SCARs. Clinical judgement is largely used in daily practice. However, it presents pitfalls, such as subjectivity and lack of standardization. Drug causality algorithms are an alternative to expert decision-making.[12]

We have used the algorithm of the ASPS[24] as the standard for a correct diagnosis, because it showed a good agreement with results of re-exposure to drugs (the true gold standard) in non-immediate hypersensitivity reactions in general (see Table 1).

Using ASPS as the standard and considering standard criteria for positivity,[15] LTT sensitivity and specificity were similar to those reported for drug allergy reactions[14,16] and anticonvulsant hypersensitivity syndrome.[37] A higher specificity, reaching values of 96%, was found when the cut-off was SI ≥2 or ≥5 in at least 2 concentrations. However, the sensitivity dropped when more stringent conditions for positivity were applied, as well as NPVs, suggesting that standard criteria should be considered for positivity.

The low values of LTT sensitivity and specificity in the acute phase analysis could suggest that performance of LTT is useless in the acute phase. Nevertheless, LTT was highly specific (90%) when SI >2 (or >5) in at least 2 drug concentrations tested was considered as a positive result, suggesting that LTT in the acute phase could be helpful to those patients for whom a management drug decision should be made as soon as possible (e.g. antituberculosis drugs). Few data regarding in vitro diagnostic tests performed during the acute phase have been published. A sensitivity of 50% and specificity of 95% were found in the acute phase of delayed-type drug hypersensitivity reactions.[17] However, patients with various clinical entities were included (only 7 patients with DRESS of 43), and clinical judgement was used as the standard for drug causality. On the other hand, several authors recommend performing the diagnostic test 4-8 weeks after the reaction.[15,23]

The ROC curve analysis also supports a better predictive ability of LTT to discriminate culprit drugs from non-culprit drugs when performed after recovery in patients with DRESS. Moreover, although an SI ≥3 had been suggested as the cut-off for beta-lactam antibiotics, the ROC curve analysis identified an SI = 1.82 as the optimal cut-off for beta-lactams in DRESS cases. This SI is similar to values considered by some authors for drugs in general.[23]

In a second analysis, considering SI ≥2 as a positive result for all drugs, the sensitivity of LTT in the recovery phase of DRESS increased for all drugs, reaching 92.3% for beta-lactams. The specificity in the analysis for all drugs decreased slightly, but it continued being 100% for beta-lactams. Moreover, the NPV, which was low when testing beta-lactam antibiotics, increased drastically from 46.2% to 75% when the threshold for positivity was lowered. It is of note that in our DRESS cases, all beta-lactam tests were positive in more than one concentration with these criteria. Differences with previous studies might rely on the phenotype of the patients analysed, which in our case were restricted to DRESS, and the nature of the drugs, which in previous studies involved mainly amoxicillin and/or penicillin-related cases,[14,16] which are medications widely used by the general population. In our series, only a minor proportion of cases were tested with amoxicillin or amoxicillin/clavulanic acid (see Figure 1).

TABLE 3 Sensitivity and specificity of LTT in the recovery phase of DRESS considering SI ≥2 as cut-off for positivity for all drugs tested

Drugs	No. of positives	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*P*-value[a]	κ (*P*)
All	At least 1	80.0	71.9	83.3	57.5	<.0001	.481 (<.0001)
	2 or more	69.4	84.4	92.2	50.9	<.0001	.447 (<.0001)
Beta-lactams	2 or more[b]	92.3	100	100	75.0	<.0001	.818 (<.0001)

[a]Fisher's exact test.
[b]All positive LTTs to beta-lactams were positive in more than 1 concentration tested.

When using re-exposure to the drug in DRESS cases as the "gold standard," LTT sensitivity was 100% and LTT specificity was 62.5% (Table 2). Among the cases analysed, 5 negative re-exposures corresponded to 5 negative LTTs, but there were 3 negative re-exposures with a positive LTT (SI >3 in more than one concentration). They corresponded to two patients in whom all the tested drugs had a high SI, but the indexes were more than fivefold higher for those drugs to which the reaction was imputed according to ASPS. For this reason, these patients were re-exposed to the drugs although the LTT was positive. False positive data have been previously reported in a few cases.[14,34] Moreover, our sample size is too small to extract strong conclusions.

Regarding comparisons with skin tests, our results are in agreement with previous reports of a higher sensitivity of LTT[14,16,19,38] and confirm that the possibility of positive ST is very low when the LTT results are negative.

It has been suggested that the sensitivity and specificity of the LTT could depend on the drug tested. Our data confirm that the LTT is an excellent tool for diagnosis of DRESS induced by anticonvulsants (see Table 2), in agreement with previous reports,[39,40] or to drugs typically involved in DRESS such as sulfamethoxazole. In our series, all anticonvulsants- or sulfamethoxazole-related DRESS cases (according to ASPS) had a positive LTT. It is of note that only one patient was tested (and only in the acute phase) with allopurinol in our series. However, the low frequency of tests performed with the usual suspects in this study does not necessarily reflect the incidence of DRESS cases induced by those drugs in our population. Beta-lactam antibiotics were the most numerous group of drugs evaluated in our series. The data suggest that LTT is also a good diagnostic tool in beta-lactam-induced DRESS, as explained above.

LTT with antituberculosis drugs showed an 87.5% sensitivity and 100% specificity. Similar specificity values and much lower sensitivity values were previously reported.[41,42] However, both groups performed LTT studies in the acute phase, which could explain the low sensitivity, and included various clinical entities. Our results in the recovery phase suggest a diagnostic role for LTT in antituberculosis drug-induced DRESS.

Although sensitivity and NPV were very good in metamizole- and vancomycin-related cases, the low specificity (high rate of false positives) indicated that positive LTT data should be considered in relationship to the clinical history in those patients. The ability of vancomycin to enhance lymphocyte proliferation in non-sensitized individuals had been reported previously.[13] Nonetheless, the sample size is small and more cases need to be analysed in order to draw strong conclusions.

Our study has several limitations, such as the use of the pharmacovigilance algorithm as an approach to the gold standard for correct diagnosis, the involvement of a single laboratory, and the small sample size regarding single drugs and acute phase analysis. Modifications of experimental conditions, such as days of culture (5-7), number of cells tested (1.5×10^5-2.5×10^5/well), type and percentage of serum or plasma (5%-20% of autologous serum or autologous plasma or AB human serum), and the technique used to estimate lymphocyte proliferation hinder the comparison of data obtained by various laboratories. However, to our knowledge, this is the largest LTT series studied specifically in DRESS, and the first to estimate sensitivity and specificity of LTT in the acute and recovery phases of DRESS.

In conclusion, our results show that LTT has a good sensitivity and specificity and is a reliable tool for diagnosis of drug causality, mainly when performed in the recovery phase in DRESS syndrome, although strong positive results in the acute reaction could be informative in some cases. The number of drugs tested in the acute and recovery phases in the same patients was too small to perform a paired analysis between acute and recovery data. Further research and analysis in acute samples would be needed to confirm this point.

This technique should at least be available in reference centres managing DRESS. We agree with other authors that a combined approach using a detailed case history, LTT, and skin tests should be used to identify the causative drug.[20]

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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SUPPORTING INFORMATION

TITLE: SENSITIVITY AND SPECIFICITY OF LYMPHOCYTE TRANSFORMATION TEST IN DRUG REACTION WITH EOSINOPHILIA AND SYSTEMIC SYMPTOMS CAUSALITY ASSESSMENT

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Table S1. Patients with DRESS included in the prospective analysis.

Patient ID	PIEL*enRed* /RegiSCAR	Sex	Age	Hospital	Nº drugs tested	Phase
ID_1	5010040	M	60	HULP	4	Recovery
ID_2	5010045	M	59	HULP	**2**	**Acute**
					3	Recovery
ID_3		M	83	HULP	4	Recovery
ID_4		M	77	HULP	1	Recovery
ID_5		F	67	HULP	1	Recovery
ID_6		F	42	HULP	3	Recovery
ID_7		F	65	HULP	4	Recovery
ID_8	5010034	F	71	HULP	5	Recovery
ID_9	5010020	F	62	HULP	1	Recovery
ID_10	5010042	F	39	HULP	**2**	**Acute**
					2	Recovery
ID_11	5010026	F	74	HULP	**2**	**Acute**
					3	Recovery
ID_12	5010031	F	73	HULP	2	Recovery
ID_13		M	61	HULP	2	Recovery
ID_14		F	76	HULP	**1**	**Acute**
ID_15		F	75	HULP	2	Recovery
ID_16		F	44	HULP	3	Recovery
ID_17	5010032	M	21	HUP/H12 Oct	6	Recovery
ID_18		F	39	HULP	1	Recovery
ID_19		F	67	HULP	3	Recovery
ID_20	5010033	M	30	HULP	**2**	**Acute**
					2	Recovery
ID_21		F	76	HULP	8	Recovery
ID_22		F	7	HULP	1	Recovery
ID_23	5010073	M	42	HULP	**1**	**Acute**
ID_24	5010071	M	28	HULP	**2**	**Acute**
					2	Recovery
ID_25		M	67	HULP	3	Recovery
ID_26		F	81	HULP	4	Recovery
ID_27		M	40	HULP	3	Recovery
ID_28		F	39	HULP	4	Recovery
ID_29		F	59	HULP	3	Recovery
ID_30		M	18	HULP	2	Recovery
ID_31		F	42	HULP	4	Recovery
ID_32	5010050	M	37	HUPA	2	Recovery
ID_33	5010046	F	77	HUGM	**7**	**Acute**
ID_34	5010055	F	82	HULP	**1**	**Acute**
ID_35	5010009	F	27	HUPH-M	**4**	**Acute**
					4	Recovery
ID_36	5010066	F	16	HUPA	1	Recovery
ID_37	5010074	M	87	HUGM	**3**	**Acute**
					3	Recovery
ID_38		M	60	HULP	1	Recovery
ID_39	5010091	M	75	HULP	3	Recovery
ID_40	5010098	M	65	HULP	7	Recovery
ID_41	5010077	F	39	HULP	**3**	**Acute**
					5	Recovery

Table S2: Drugs used in LTT: Solvents and maximal concentrations.

Drug	Nº tests	Solvent	Highest concentration# in LTT assay	Nº positive* tests
Allopurinol	1	NaOH 1 M	100 µg/mL	1
Amoxicillin	4	Intravenous	500 µg/mL	4
Amoxicillin-Clavulanic Acid	3	Intravenous	500 µg/mL	3
Aztreonam	1	Intravenous	1000 µg/mL	0
Bisoprolol	1	RPMI	2.5 µg/mL	1
Buscapine	1	Intravenous	20 µg/mL	0
Carbamazepine	2	DMSO	100 µg/mL	2
Carvedilol	2	DMSO	1 µg/mL	0
Cefepime	3	Intravenous	200 µg/mL	2
Cefixime	2	MetOH	200 µg/mL	0
Ceftriaxone	6	Intravenous	200 µg/mL	3
Cefuroxime	1	Injectable	200 µg/mL	0
Cetirizine	2	RPMI	5 µg/mL	1
Clindamycin	3	Intravenous	100 µg/mL	1
Cloxacillin	2	Intravenous	250 µg/mL	1
Dextromethorphan	1	MetOH	1 µg/mL	0
Enalapril	1	RPMI	1 µg/mL	1
Ertapenem	1	Intravenous	500 µg/mL	0
Ethambutol	4	Intravenous	100 µg/mL	1
Etoricoxib	1	DMSO	50 µg/mL	1
Phenytoin	2	Intravenous	100 µg/mL	2
Ferroglycine Sulphate	1	Oral drops	50 µg/mL	1
Fluconazole	3	Intravenous	100 µg/mL	1
Gabapentin	1	RPMI	10 µg/mL	1
Gentamycin	1	Intravenous	50 µg/mL	0
Ibuprofen	2	Intravenous	50 µg/mL	0
Isoniazid	4	Intravenous	100 µg/mL	4
Lamotrigine	2	DMSO	50 µg/mL	2
Lansoprazole	1	RPMI	5 µg/mL	1
Levetiracetam	2	Intravenous	100 µg/mL	0
Levodopa	1	HCl 0,1 N	25 µg/mL	0
Levofloxacin	4	Intravenous	50 µg/mL	2
Lorazepam	1	DMSO	25 µg/mL	1

Meropenem	3	Intravenous	100 µg/mL	0
Metamizole	10	Intravenous	50 µg/mL	8
Metronidazole	1	RPMI	100 µg/mL	0
Naproxen	2	EtOH	100 µg/mL	1
Natalben Supra®	2	RPMI	10^{-3} dilution	2
Omeprazole	1	Intravenous	50 µg/mL	0
Pantoprazole	1	Intravenous	50 µg/mL	0
Paracetamol	5	Intravenous	50 µg/mL	1
Pyrazinamide	4	RPMI	100 µg/mL	1
Pseudoephedrine	1	RPMI	10 µg/mL	0
Rifampicin	4	Intravenous	100 µg/mL	2
Sulfamethoxazole	2	Intravenous	100 µg/mL	2
Sulfamethoxazole/trimethoprim	2	Intravenous	100 µg/mL	2
Spironolactone	1	DMSO	2.5 µg/mL	1
Piperacillin/tazobactam	16	Intravenous	500 µg/mL	9
Ticagrelor	1	Dimethylformamide	10 µg/mL	1
Tigecycline	1	Intravenous	1 µg/mL	0
Trimethoprim	1	Intravenous	25 µg/mL	1
Vancomycin	11	Intravenous	50 µg/mL	7
Visipaque (iodinaxol)	1	Intravenous	10 mg/mL	1
Vitamin B1	1	Intravenous	100 µg/mL	1
Vitamin B12	1	Intravenous	5 µg/mL	0
Vitamin B6	1	Intravenous	100 µg/mL	1
Vitamin B9	1	Intravenous	20 µg/mL	0

[#]Concentrations were used according to previously published data. When no previous data were available, preliminary assays were performed in healthy donors starting with 1/500 dilutions of drug stock preparations. Only non-toxic concentrations (SI >0.7 in healthy donors) were used.
*Considering standard criteria for LTT positivity

Table S3.

Algorithm of the Spanish Pharmacovigilance System

1. The chronology referred to as the interval between drug administration and effect:

1. Compatible (score +2)
2. Not fully supported (+1)
3. No information (0)
4. Incompatible chronology (-1)
5. Particular case of a withdrawal syndrome (+2).

2. The literature, defining the degree of knowledge of the relationship between the drug and the effect:

1. Known in the reference literature (+2): collected in Summary of Product Characteristics, or the adverse drug reaction was found in clinical trials, or the association was found in cohort studies or in case-control studies.
2. Occasionally known (+1): only found in published case reports.
3. Unknown (0)
4. Unrelated to the drug (-1): presence of confounding variables. Confounding variables occur when the estimate of a measure of association between the drug exposure and health status is distorted by the effect of one or several other variables that are also risk factors for the outcome of interest.

3. The evaluation of drug withdrawal:

1. Withdrawn with improvement (+2)
2. Withdrawn with no improvement (-2)
3. Not withdrawn, effect did not improve (+1)
4. Not withdrawn, effect improved (-2)
5. No information (0)
6. Death or irreversible effect (0)
7. Not withdrawn, but there was a tolerance effect (+1)
8. Not withdrawn, improved with symptomatic treatment (+1)

4. The rechallenge effect:

1. Positive, effect reappearance (+3)
2. Negative, the effect does not reappear (-1)
3. No re-exposure or no information (0)
4. Death or irreversible effect (0)
5. Positive for a different specialty with the same active ingredient or parent drug (+1)

5. Alternative causes:

1. Yes, a pathology or other drugs (-3)
2. Similar likelihood for drug and other causes (-1)
3. Missing information (0)
4. There is not any alternative cause (+1)

The total score regarding the probability is established according to the following 5 categories:

- **Unclassifiable (missing data)**
- **Unlikely (<0)**
- **Conditional (1–3)**
- **Possible (4–5)**
- **Probable (6–7)**
- **Defined (>8)**

Table S4. Contingency table: Sensitivity and specificity of skin tests in DRESS using ASPS as the gold standard.

	ASPS		
Skin tests	Related drugs (ASPS ≥4)	Nonrelated drugs (ASPS ≤ 3)	**Sum**
Positive	**17**	1	18
Negative	31	**12**	43
Sum	48	13	**61**

p-value=.085 (Fisher's exact test); p-value=.052 (χ^2 test)
Cutaneous tests: sensitivity = 35.4%; specificity = 92.3%; PPV = 94.4 %; NPV = 27.9%; κ= .151.

Table S5. Comparison of results of LTT and skin tests in DRESS.

	Skin tests		
LTT	Positive	Negative	**Sum**
Positive	**14**	21	35
Negative	4	**22**	26
Sum	18	43	**61**

p=.049 (Fisher's exact test)
LTT sensitivity = 77.8%; specificity = 51.2%; PPV = 40 % NPV = 84.6%
κ = .253.

Table S6. Sensitivity and specificity of LTT in DRESS cases in which skin tests were performed.

	Classification ASPS		
LTT	Related drugs (ASP≥4)	Nonrelated drugs (ASP≤ 3)	**Sum**
Positive	**38**	2	40
Negative	16	**15**	31
Sum	54	17	**71**

p<.0001 (Fisher's exact test)
LTT sensitivity = 70.4%; specificity = 88.2%; PPV = 95%; NPV = 48.4%;
κ = .457.

Delay to acute test

Patient ID_2 — 12 days

Drug	ASPS	LTT acute	LTT recovery	ST
Cetirizine	Conditional	Positive*	Positive	ND
Amoxicillin	Probable	Negative	Positive	ND
Pseudoephedrine	Conditional	ND	Negative	PT -

Patient ID_10 — 2 days

Drug	ASPS	LTT acute	LTT recovery	ST
Pipe / Tazo	Probable	Positive	Positive	IDT+
Vancomycin	Conditional	Negative	Positive	IDT+

Patient ID_11 — 6 days

Drug	ASPS	LTT acute	LTT recovery	ST
Cefepime	Probable	Negative	Positive	ND
Vancomycin	Probable	Negative	Positive	ND
Fluconazole	Unlikely	ND	Positive	ND

Patient ID_20 — 10 days

Drug	ASPS	LTT acute	LTT recovery	ST
Amoxicillin	Defined	Positive	Negative [§]	IDT+
Metamizole	Defined	Positive	Positive	IDT+

Patient ID_24 — 4 days

Drug	ASPS	LTT acute	LTT recovery	ST
Pipe / Tazo	Probable	Negative	Positive	ND
Clindamycin	Possible	Negative	Negative	ND

Patient ID_35 — 12 days

Drug	ASPS	LTT acute	LTT recovery	ST
Rifampicine	Possible	Negative	Negative	PT-
Ethambutol	Unlikely	Negative	Negative	ND
Isoniazide	Probable	Positive	Positive	PT +
Pirazinamide	Probable	Negative	Negative	PT-

Patient ID_37 — 7 days

Drug	ASPS	LTT acute	LTT recovery	ST
Pipe / Tazo	Possible	Negative	Negative	PT - IDT -
Meropenem	Possible	Negative	Negative	PT - IDT -
Vancomycin	Probable	Negative	Positive	PT -

Patient ID_41 — 8 days

Drug	ASPS	LTT acute	LTT recovery	CT
Natalben Supra®	Possible	Positive	Positive	ND
Buscapine	Unlikely	Negative	ND	ND
Vitamin B9	Probable	Negative	ND	ND
Ferroglycine	Probable	ND	Positive	PT-
Vitamin B1	Probable	ND	Positive	PT - IDT +
Vitamin B6	Probable	ND	Negative	IDT -
Vitamin B12	Probable	ND	Negative	PT - IDT +

ASPS: Algorithm of the Spanish Pharmacovigilance System; LTT: Lymphocyte transformation test; ND: No data; ST: Skin tests: PT: Patch test; IDT: Intradermal test;
Pipe / Tazo: Piiperacillin /Tazobactam

*LTT was considered positive according to standard criteria (stimulation index [SI] ≥2), except for betalactam antibiotics (SI≥3)
[§]Negative with standard criteria. SI>2.5 in three of the concentrations tested

18 Drugs tested in Acute and Recovery phases

ACUTE PHASE RESULTS:

	Related (ASPS≥4)	Non related (ASPS<4)	Sum
LTT pos	5	1	6
LTT neg	10	2	12
Sum	15	3	18

Sensitivity: 33.3%; Specificity 66.7%
PPV: 83.3%; NPV: 16.7%
Fisher's exact test: not significant

RECOVERY PHASE RESULTS:

	Related (ASPS≥4)	Non related (ASPS<4)	Sum
LTT pos	11	2	13
LTT neg	4	1	5
Sum	15	3	18

Sensitivity: 73.3%; Specificity 33.3%
PPV: 84.6%; NPV: 20%
Fisher's exact test: not significant

Figure S1. Eight DRESS cases were tested in the acute and recovery phases. In total, 26 drugs were tested in these patients. Results in the acute and recovery phases, as well as ST, are shown for comparative purposes. Contingency tables showing sensitivity and specificity of the LTTs performed with the 18 drugs tested in both the acute and recovery phases are shown.

Delay from onset to test

Patient ID_14

Drug	ASPS	LTT acute	ST	
Levofloxacin	Possible	Positive*	ND	**8 days**

Patient ID_23

Drug	ASPS	LTT acute	ST	
Levetiracetam	Probable	Negative	ND	**2 days** (LTT positive 2 years later)

Patient ID_34

Drug	ASPS	LTT acute	ST	
Piperacillin / Tazobactam	Probable	Negative	ND	**2 days**

Patient ID_33

Drug	ASPS	LTT acute	ST	
Ceftriaxone	Unlikely	Positive	ND	**5 days**
Lansoprazol	Unlikely	Positive	ND	
Levofloxacine	Unlikely	Positive	ND	
Espironolactona	Unlikely	Positive	ND	
Bisoprolol	Unlikely	Positive	ND	
Alopurinol	Probable	Positive	ND	

ASPS: Algorithm of the Spanish Pharmacovigilance System; LTT: Lymphocyte transformation test; ND: No data; ST: Skin tests

*LTT was considered positive according to standard criteria (stimulation index [SI] ≥2), except for betalactam antibiotics (SI≥3)

Figure S2. Results from the 4 DRESS cases that were tested only during the acute phase.