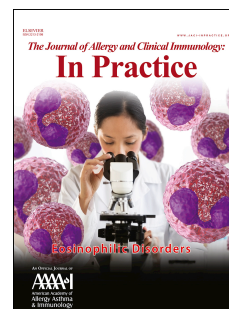


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The role of *in vivo* and *ex vivo* diagnostic tools in severe delayed immune-mediated adverse antibiotic drug reactions

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Abstract

Background

The use of *in vivo* and *ex vivo* diagnostic tools for delayed immune-mediated adverse drug reactions (IM-ADR) is currently ill defined.

Objective

To determine if the combination of skin testing and/or interferon- γ (IFN- γ) enzyme-linked immunoSpot assay (ELISpot) can aid diagnosis of these allergy phenotypes.

Methods

Patients with antibiotic-associated severe delayed IM-ADR hypersensitivity, including SJS and TEN, DRESS, AGEP, GBFDE and severe MPE, were prospectively recruited. *In vivo* testing was completed to the implicated drug(s) and *ex vivo* testing was performed with the patient's PBMC stimulated with the relevant antibiotic concentrations for IFN- γ release ELISpot measurement.

Results

Eighty-one patients met the inclusion with DRESS (42; 51.9%) accounting for the majority of cases. Among the 63 (78%) who had an ELISpot assay performed, 34 (54%) were positive to at least one implicated antibiotic (median spot forming units/ million cells, 99.5; [IQR], 68-187) with glycopeptide being a strong predictor of positivity (aOR 6.11 95% CI 1.74, 21.42). In combination (*in vivo* and *ex vivo*), 51 (63%) of those tested were positive to an implicated antibiotic. For DRESS and severe MPE associated with penicillins and cephalosporins, this

combination confirmed the culprit agent in 11/12 of the cases and in 6/7 for DRESS associated with glycopeptides.

Conclusion

This study demonstrates that using *in vivo* in combination with *ex vivo* testing can enhance the diagnostic approach in these severe phenotypes by assisting with the identification of possible culprit antibiotics.

Highlights box:

1. What is already known about this topic?

The use of *ex vivo* diagnostic tools for delayed immune-mediated adverse drug reactions has the advantage of carrying no risk of drug re-exposure for the patient but is not available for routine diagnostic use in most centers.

2. What does this article add to our knowledge?

This study shows that a combination of *in vivo* (skin testing) and *ex vivo* (IFN- γ release ELISpot) diagnostic tools in severe delayed immune-mediated antibiotic adverse drug reactions improves causality assessment, in particular for DRESS glycopeptide associated reactions.

3. How does this study impact current management guidelines?

Using *in vivo* in combination with *ex vivo* testing can enhance the diagnostic approach in severe phenotypes by assisting with the identification of possible culprit antibiotics.

Key words:

107 Severe cutaneous adverse reaction (SCAR); delayed hypersensitivity; T-cell mediated
108 hypersensitivity; adverse drug reaction (ADR); skin testing; intradermal testing; interferon- γ
109 enzyme-linked immunoSpot assay; *in vitro* diagnostic; *ex vivo* diagnostic.

110

111 Abbreviations:

- 112 AGEF - acute generalized exanthematous pustulosis
- 113 ALDEN - algorithm for assessment of drug causality for epidermal necrolysis
- 114 aOR - adjusted odds ratio
- 115 Delayed IM-ADR - delayed immune-mediated adverse drug reaction
- 116 DRESS - drug reaction with eosinophilia and systemic symptoms
- 117 ELISpot - enzyme-linked immunoSpot assay
- 118 GBFDE - generalized bullous fixed drug eruption
- 119 IDT - intradermal testing
- 120 IFN- γ - interferon- γ
- 121 MPE - maculopapular exanthema
- 122 PBMC - peripheral blood mononuclear cells
- 123 PT - patch testing
- 124 RegiSCAR - registry of severe cutaneous adverse reaction
- 125 SCAR - severe cutaneous adverse reaction
- 126 SFU - spot forming units
- 127 SJS - Stevens-Johnson syndrome
- 128 TEN - toxic epidermal necrolysis

Introduction

Delayed hypersensitivities are a group of presumed T-cell mediated adverse drug reactions (ADRs) that vary from isolated benign skin conditions to severe cutaneous adverse reactions (SCARs) that are associated with significant acute and long-term morbidity and mortality (1). *In vivo* skin testing, such as patch testing (PT) and delayed intradermal testing (IDT), diagnostic approaches to severe delayed immune-mediated ADRs (delayed IM-ADR) are limited by availability and variable sensitivity (2, 3). Functional *ex vivo* assays include cytokine release enzyme-linked immunoSpot (ELISpot) assay, an emerging diagnostic tool which uses cytokine release, typically IFN- γ , after the patient's peripheral blood mononuclear cells (PBMC) are stimulated with the suspected drug(s). This diagnostic tool, while having the advantage of carrying no risk of drug re-exposure for the patient, is not available for routine diagnostic use in most centers. The ELISpot assay has shown promise (4-6), although its role in the diagnostic approach to delayed IM-ADR is ill defined. We sought to examine the utility of *in vivo* and *ex vivo* testing using the IFN- γ ELISpot assay to determine drug causality in a cohort of patients with antibiotic-associated delayed IM-ADR.

Methods

Patients with antibiotic-associated delayed IM-ADRs, including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug reaction with eosinophilia and systemic symptoms (DRESS), acute generalized exanthematous pustulosis (AGEP), generalized bullous fixed drug eruption (GBFDE) and severe maculopapular exanthema (MPE), were prospectively recruited from a tertiary referral drug allergy testing centre between 1st March 2017 and 21st July 2020 at the Austin Health Hospital and Peter MacCallum Cancer Centre Melbourne, Australia. Further, cases may have been co-recruited to the Australasian Registry of Severe Cutaneous Adverse Reactions (AUS-SCAR) – a prospective national study (ACTRN12619000241134) and some of the cases might have been included in a previous publication (7). The patients had a Naranjo ADR score (8) of 5 or more and scored a minimum in the algorithm of drug causality for epidermal necrolysis (ALDEN) score (9) of 4 or more for SJS and TEN; Registry of Severe Cutaneous Adverse Reaction (RegiSCAR) score (10) of 4 or more for DRESS ; AGEP score (11) of 2 or more for AGEP. Severe MPE was defined as an extensive cutaneous exanthem with more than 50% of body surface area and RegiSCAR score of 2 to 3 (possible), as previously defined (7).

Patients were excluded based on previously published criteria, including an alternative viral, bacterial, or autoimmune etiology, evidenced by any one of the following: (1) positive polymerase chain reaction for herpesvirus (HSV1/2), cytomegalovirus, Epstein-Barr virus or enterovirus, (2) positive *Mycoplasma* species on polymerase chain reaction (respiratory specimen or serology), or (3) detectable antinuclear antigen antibody titer of more than 1:64 (4). Patients were also excluded if their clinical picture was consistent with an alternative diagnosis

or if the skin biopsy (histopathology or direct immunofluorescence) was not consistent with a drug reaction (4).

In vivo testing (PT or IDT) was performed to the implicated drug(s) at the clinician's discretion using previously published non-irritating concentrations (12, 13). IDT was performed at least 6 weeks following the resolution of cutaneous manifestations, as per previous publications (4).

The *ex vivo* testing was performed in triplicate with the patient's PBMC stimulated with the relevant antibiotic concentrations in order to measure the CD4 and CD8 T-cell dose dependent IFN- γ release via ELISpot (4). Antibiotics used in the IFN- γ release ELISpot assay were sourced from local pharmacy if available in commercial intravenous formulation or in a powder form with subsequent dilution in the laboratory setting, depending on their solubility. PBMCs (200,000 cells per well) were incubated with investigated antibiotics at concentrations representative of peak serum concentrations (*C_{max}*) and a level 10-fold higher than *C_{max}*. Testing was also performed with a negative (unstimulated) and positive control (anti-CD3 antibody; Mabtech®, Australia) in duplicate and healthy control (penicillin-exposed nonallergic) for all testing antibiotic concentrations. IFN- γ secretion was captured by the anti-IFN- γ antibody 18-24 hours following antibiotic addition to patient PBMC, with detection antibody and enzyme substrate being added just before plate reading. The spot forming units (SFU) representing cells that secrete IFN- γ were then identified and counted automatically with an AID ELISpot Reader (software version 7.0) (Mabtech®). A positive response was defined as equal or greater than 50 SFU/million cells after background (unstimulated control) removal as per previously published definitions (14, 15). Five tolerant controls, defined as patients assessed in the outpatient clinic

that had tolerated a complete course (median treatment duration 10 days) of the antibiotic used for ELISpot stimulation, were included in the study.

Descriptive results are presented as frequency (percentage) and median (interquartile range). Sensitivity was calculated for each diagnostic test and phenotype. Fisher's Exact test was used for comparison between phenotypes. Logistic regression was used to determine predictors of a positive diagnostic test (separately for *in vivo* and *ex vivo*; predictors: age, sex, immunosuppression, phenotype, latency and culprit drug). Mixed effects logistic regression was used to compare *in vivo* positivity against *ex vivo* test positivity within the main subgroups of reaction type and antibiotic. Variables with $p < 0.100$ were included in the multivariable model. Statistical analyses were performed with Stata/IC 16.1 and figures representing ELISpot results were generated using GraphPad Prism (7.0a, Macintosh version). The study was approved by Austin Health Human Research Ethics Committee (HREC/15/AUSTIN/75 and HREC/50791/Austin-19) and the investigators obtained written informed consent from the participants.

Results

Baseline demographic. Eighty-one patients with an antibiotic-associated delayed IM-ADR met the inclusion and exclusion criteria (**Figure 1**). Their clinical characteristics, the clinical investigations and the implicated antibiotics are summarized in **Table 1, Table 2, and Table 3**, respectively. The phenotypes encountered were DRESS (42; 51.9%) severe MPE (25; 30.9%), AGEP (6; 7.4%), SJS/TEN (7; 7.9%) and GBFDE (1; 1.2%) (**Figure 1**). The most common implicated antibiotics for DRESS were penicillins (24/42; 57.1%) and glycopeptides (22/42; 52.4%) (**Table 3**). Based on medical records review, penicillins were considered causal in a majority of SJS (4/6; 66.7%) and severe MPE (20/25; 80%) cases and in all AGEP cases (6/6) (**Table 3**).

Skin testing results (*in vivo*). The IDT and PT results per phenotype are summarized in **Table 2**. Among the 81 patients included, 68 (84%) underwent IDT and/or PT. The main reasons for not performing *in vivo* testing was patient refusal or underlying skin scarring or hypopigmentation in the areas most appropriate for skin testing. Of those skin tested, there was a non-statistically significant higher rate of skin test positivity in DRESS compared with SJS/TEN phenotypes, although based on small SJS/TEN numbers (14/22 vs 0/2, $p=0.163$) (**Table 2**). The median skin test latency for the positive tests ($N = 33$) was 121 days (IQR, 85 - 422). No predictors of a positive skin test (PT or IDT) were identified (**Table E2**).

One patient (0.01%) with DRESS phenotype presented a mild systemic reaction (fever and mild generalized skin eruption, but no evidence of DRESS relapse) 48 hours after intradermal skin testing to beta-lactams that was performed 7 weeks after the acute phenotype. No patients had a relapse of their severe delayed IM-ADR.

IFN- γ release ELISpot assay responses (*ex vivo*). The IFN- γ release ELISpot assay was performed in 78% of the final cohort (63/81) and 34/63 (54%) were positive to at least one implicated antibiotic (median SFU/ million cells, 99.5; interquartile range [IQR], 68-187) (**Figure E1**). Nineteen (56%) were positive to more than one antibiotic, 18 (53%) were only positive to the highest antibiotic concentration tested, and 15 (44%) were positive to both concentrations of the antibiotics tested. The median PBMC collection latency for the positive tests (N = 34) was 133 days (IQR, 32 - 401).

The IFN- γ release ELISpot assay results for all tested antibiotics in patients with SCAR are represented in **Figure E1** and the results stratified by phenotype are illustrated in **Table 2**. There was no difference in *ex vivo* positivity with severe MPE compared with DRESS (9/17 (53%) vs 19/34 (56%); $p = 1.00$) and DRESS compared with SJS phenotypes (19/34 (56%) vs 3/5 (60%); $p = 1.00$). The sensitivity of IFN- γ release ELISpot assay for DRESS was 56%. The specificity can be calculated at 100%, when considering the absence of false positive results in the tolerant control group (**Figure E2**).

Using the multivariable model, a glycopeptide implicated in causality was a strong predictor of IFN- γ ELISpot test positivity (aOR 4.80, 95% CI 1.48, 15.54) (**Table E3**). Further, younger age was also noted to be possibly associated with positive IFN- γ ELISpot result (aOR 0.97, 95% CI 0.95, 0.99, p -value=0.041). None of the tolerant controls exhibited a positive result (median SFU/million cells, 0; IQR, 0-1.3) (**Figure E2**).

Agreement and utility of combined *in vivo* and *ex vivo* testing. In the final cohort, 63% (51/81) were positive to an implicated antibiotic on either *in vivo* or *ex vivo* testing and 20%

(16/81) were positive on both investigations (**Table 2**). The median latency to testing for *in vivo* testing was 129 days and 115 days for *ex vivo* testing (**Table 1**).

In those positive on both modalities, the same drug(s) was implicated in 13/16 (81%) of patients.

In two patients, there were discrepant results in the *in vivo* and *ex vivo* assays. In these patients, the implicated drugs shared the same antibiotic class and shared the same penicillin beta-lactam ring. In another case (1/16) the drug classes were different but components of the same antibiotic (*ex vivo* positive for sulfamethoxazole and PT was positive for trimethoprim).

For the most commonly encountered phenotype, DRESS, 62% (26/42) patients had a positive *in vivo* and/or *ex vivo* test result (**Table 2**).

The overall sensitivity for combination testing of skin testing and ELISpot was 63%. Among the 22 patients with glycopeptide DRESS, 15/21 (71.4%) were positive to ELISpot and 7/8 (87.5%) on IDT/PT (**Table E4**). For the 21 patients with a penicillin-cephalosporin associated severe MPE, 7/13 (53.8%) were positive on ELISpot and 14/15 (93.3%) on *in vivo* testing (**Table E4**).

The combination of *ex vivo* and *in vivo* testing confirmed the allergic diagnostic in 11/12 of the cases for DRESS and severe MPE associated with penicillins and cephalosporins and 6/7 for DRESS associated with glycopeptides (**Table E4**). When compared with *in vivo* testing, *ex vivo* testing seems to be associated with more positive results for DRESS, while, on the contrary, skin testing performed better for severe MPE; however due to small sample size these results were not statistically significant (**Table E5**).

Discussion

We present one of the largest cohorts of antibiotic-associated delayed IM-ADR, using combined skin testing and the novel IFN- γ release ELISpot assay, building on a previously published pilot study (4). The ability to identify the culprit antibiotic has a significant clinical impact because multiple antibiotics are implicated in patients with SCAR (38% in our cohort). Without precise identification of the aetiological agent, these patients could potentially have to avoid multiple antibiotic classes resulting in limited therapeutic options for current and future severe infections.

In vivo or *ex vivo* testing was positive in 63% of the cases, with the highest sensitivity in the severe MPE phenotype (17/25 or 68%), which is similar to previous reports in this cohort of delayed IM-ADR, albeit in potentially non-severe phenotypes (16, 17). Skin testing was positive in 49% of patients, consistent with the range previously reported (6.6-36.3% for MPE and 64-100% for DRESS (3))

IFN- γ release ELISpot contributed to the diagnostic results in 63% of patients, picking up an additional 15 (19%) cases that were missed with skin testing alone (**Table E1**). The sensitivity of IFN- γ release ELISpot assay for DRESS was 56%. Our group has previously demonstrated a sensitivity of 52% in patients with SCAR and specificity of 100% (4). In a cohort of 22 patients with amoxicillin MPE, the sensitivity of the IFN- γ ELISpot was 91% (15/22) when the cut-off were more than 30 SFU/10⁶ (16). Using this same reference value, the sensitivity was 87.5% (7/8) in a study involving 8 patients with hypersensitivity to piperacillin (18). Overall, these data demonstrate a reproducible sensitivity and specificity that can be used as an adjunct to clinical assessment, allowing practice improvement. Although we did not observe any instances where *in*

vivo testing precipitated a recurrence of SCAR, the IFN- γ release ELISpot assay is without clinical risk and appears to have a good specificity, and, in the most part, has good agreement with positive *in vivo* (skin testing), when performed. This is also relevant as skin testing in the setting of DRESS should ideally be avoided in the 3 to 6 months period following the acute syndrome because of the risk of coincident DRESS relapse. We demonstrate that the likelihood of a positive IFN- γ ELISpot result is higher in glycopeptide associated delayed IM-ADR and in younger patients – aiding future patient selection. In our cohort, the combination of IFN- γ ELISpot and skin testing for severe MPE and DRESS allowed us to identify a culprit drug in 43/67 (64.2%) of the patients tested. When comparing *in vivo* with *ex vivo* testing, IFN- γ ELISpot seems to be a more valuable tool for DRESS phenotype while skin testing provided more positive results for severe MPE. The results are limited by the small study numbers and with non-statistically significant confidence intervals and p-values (**Table E5**).

We propose that IFN- γ release ELISpot can potentially be performed when *in vivo* testing is considered unsafe or is not available to clinicians and where PMBCs could be sent to a centralized specialized immunology laboratory.

Limitations of this study include the single regional experience in a predominantly Caucasian population, the long latency period between the reaction date and the assessment date, and the absence of universal drug challenge or provocation for patients who had negative *in vivo* and *ex vivo* testing.

Future directions that will be helpful to study and improve the negative and positive predictive values of combination *in vivo* and *ex vivo* approaches to antibiotic associated delayed IM-ADR

include challenge studies to individual implicated antibiotics in those negative on combined *in vivo* and *ex vivo* testing, following careful risk-benefit assessment. Using a different cytokine output for the ELISpot assay based on phenotype (e.g. DRESS – IL-5, AGEP – IL-8) and further examination of the impact of latency period (acute versus convalescent bleed) may improve the diagnostic utility. Furthermore, using HLA testing for known risk alleles (e.g. HLA-A*32:01 for vancomycin) and others as these become available may be a viable approach to aid in risk stratification and diagnosis, particularly in the case of antibiotic associated delayed IM-ADR occurring in the setting of treatment with multiple antibiotics (19).

We demonstrate the potential utility of novel *ex vivo* diagnostics to supplement traditional skin testing approaches in severe antibiotic allergy. Large collaboration international networks are required to develop and standardize *in vivo* diagnostic approaches such as IDT and PT combined with *ex vivo* laboratory approaches and other approaches such as genetic testing to improve medication safety post severe delayed IM-ADR. In the interim, this study confirms that the use of IFN- γ release ELISpot for antibiotic-associated delayed IM-ADR appears a useful adjunct to standard clinical approaches in severe drug hypersensitivity where diagnostic approaches have been traditionally limited.

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TABLE 1: Baseline characteristics of patients

Characteristics	Patients (N = 81)
Age (years), median (IQR)	56 (31,85)
Sex (F), N (%)	43 (53%)
Caucasian, N (%)	69 (85%)
Immunocompromised ^a , N (%)	33 (41%)
Pre-existing skin disease or medical comorbidities, N (%)	Cardiovascular disease ^b (5; 6.2%) CPD (1; 1.2%) Diabetes mellitus ^c (3; 3.7%) Renal disease ^d (4; 4.9%) Neurological disease ^e (1; 1.2%) Psychiatric history (6/77; 7.8%)
Age-adjusted CCI, median (IQR)	5 (3,6)
Multiple implicated antibiotics, N (%)	31 (38%)
Multiple implicated drugs, N (%)	17 (21%)
Number of allergy labels, median (IQR) ^f	2 (1,3)
Skin test latency (days) median, (IQR) ^g	129 (84.5, 416)
PBMC collection latency (days) median (IQR) ^g	115 (27, 400)

Abbreviations: IQR, interquartile range; F, female; CCI, Charlson comorbidity index; CPD, chronic pulmonary disease; PBMC, peripheral blood mononuclear cells.

^a The immunocompromised category includes patients that are known for any of the following conditions: transplant recipient, hematological or oncological malignancy (in the last 5 years), corticosteroid use of more than 10 mg prednisolone equivalent per day, connective tissue or autoimmune condition and acquired immunodeficiency syndrome.

^b Cardiovascular disease includes myocardial infarct, congestive heart failure and peripheral vascular disease.

^c Diabetes mellitus includes confirmed disease with or without end organ damage.

^d Renal disease includes moderate or severe renal disease.

^e Neurological disease represents cerebrovascular disease.

^f The number of allergy labels refers to the pre-existing number of allergy labels prior allergy testing.

[§] Skin test latency and PBMC collection latency represent the time from onset of delayed immune-mediated adverse drug reaction to skin testing being performed (“skin test latency”) or PBMC collected (“PBMC collection latency”). These variables were measured in days.

TABLE 2: Summary of clinical investigations based of phenotype (N = 81)

Phenotype	Number patients N (%)	ICH – Status or Immunosuppression ^a N/ N total (%)	Ex vivo positive N/ N total (%)	IDT positive N/ N total (%)	PT positive N/ N total (%)	Any test positive N/ N total (%)
DRESS	42 (51.9%)	14/42 (33.3%)	19/34 (55.8%)	14/35 (40%)	0/9 (0%)	26/42 (61.9%)
Severe MPE	25 (30.9%)	14/25 (56%)	9/17 (52.9%)	14/24 (58.3%)	1/4 (25%)	17/25 (68%)
AGEP	6 (7.4%)	0/6 (0%)	2/5 (40%)	3/6 (50%)	0/1 (0%)	4/6 (66.7%)
SJS	6 (7.4%)	4/6 (66.7%)	3/5 (60%)	0/3 (0%)	0/3 (0%)	3/6 (50%)
GBFDE	1 (1.2%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/0 (0%)	1/1 (100%)
TEN	1 (1.2%)	1/1 (100%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/1 (0%)

Abbreviations: AGEP, acute generalized exanthematous pustulosis; DRESS, drug reaction with eosinophilia and systemic symptoms; GBFDE, generalized bullous fixed drug eruption; ICH, immunocompromised host; IDT, intradermal testing; MPE, maculopapular exanthema; PT, patch testing; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

^a The immunocompromised host includes patients that are known for any of the following conditions: transplant recipient, hematological or oncological malignancy (in the last 5 years), corticosteroid use of more than 10 mg prednisolone equivalent per day, connective tissue or autoimmune condition and acquired immunodeficiency syndrome.

TABLE 3: Implicated antibiotics based on phenotype

Phenotype	Number patients N (%)	Penicillins ^a N (%)	Cephalosporins ^b N (%)	Carbapenems ^c N (%)	Glycopeptides ^d N (%)	Other ^e N (%)
DRESS	42 (51.9%)	24 (57.1%)	16 (38.1%)	4 (9.5%)	22 (52.4%)	14 (33.3%)
Severe MPE	25 (30.9%)	20 (80%)	8 (32%)	2 (8%)	3 (12%)	8 (32%)
AGEP	6 (7.4%)	6 (100%)	3 (50%)	0 (0%)	0 (0%)	1 (16.7%)
SJS	6 (7.4%)	4 (66.7%)	2 (33.3%)	0 (0%)	0 (0%)	3 (50%)
GBFDE	1 (1.2%)	1 (100%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
TEN	1 (1.2%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)

Abbreviations: AGEP, acute generalized exanthematous pustulosis; DRESS, drug reaction with eosinophilia and systemic symptoms; GBFDE, Generalized bullous-fixed drug eruption; MPE, maculopapular exanthema; SCAR, severe cutaneous adverse reaction; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

^a The penicillins category includes amoxicillin, ampicillin, penicillin G, penicillin VK, flucloxacillin, dicloxacillin, piperacillin/tazobactam and amoxicillin/clavulanate.

^b The cephalosporins category includes cephalexin, ceftriaxone, cefepime and ceftazidime.

^c The carbapenems category includes meropenem.

^d The glycopeptide category includes vancomycin and teicoplanin.

^e The other category includes allopurinol, trimethoprim, sulfamethoxazole, ciprofloxacin, clindamycin and rifampicin.

FIGURE 1. Flow chart of patient phenotypes, inclusion and exclusion criteria

Abbreviations: AGEP, acute generalized exanthematous pustulosis; AIN, allergic interstitial nephritis; DRESS, drug reaction with eosinophilia and systemic symptoms; ELISpot, enzyme-linked immunoSpot; FDE, fixed drug eruption; GBFDE, generalized bullous-fixed drug eruption; IDT, intradermal testing; IFN- γ , interferon gamma; MPE, maculopapular exanthema; PT, patch testing; SCAR, severe cutaneous adverse reaction; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

Notes:

1) In the patients reporting multiple allergy labels, we have reported the labels where timing was confirmed (excluding the unspecified or unknown delay) as well as the labels for delayed reactions (excluding the immediate label). If a patient presented multiple delayed reactions, we have indicated the most severe reaction.

2) In terms of skin testing, for the drug available in an intravenous formulation, IDT was favoured for the non-SJS/TEN phenotypes. PT was performed only for the SJS/TEN phenotypes and when the drugs were not available in an intravenous formulation.

3) Concerning the ex vivo testing, patients recruited to participate in this study are requested to give a reasonable amount of blood for peripheral blood mononuclear cells (PBMC) extraction. Some of the patients refused to give blood and, for some patients, the PBMC cell viability was insufficient for ELISpot assay performance.

