

In vitro/ex vivo assays for severe cutaneous drug reactions



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Severe cutaneous adverse reactions (SCARs) such as acute generalized exanthematous pustulosis (AGEP), drug reaction with eosinophilia and systemic symptoms (DRESS), and severe skin necrosis and blistering as seen in Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) are presumed to be T-cell-mediated reactions. These occur most commonly in response to a drug precipitant. However, because of the morbidity and mortality of SCAR reactions, *in vivo* diagnostics (eg, skin testing) have traditionally been deployed in only a few international centers. In contrast, *ex vivo/in vitro* laboratory-based assays have existed predominantly in the research-only domain. This Paradigms and Perspectives article describes the importance of *in vivo* and *ex vivo* diagnostics to ascertain drug causality in SCAR. In this context, we summarize recently published studies and underline the importance of international collaboration for *in vivo* and *ex vivo* diagnostics in SCARs.

IN VIVO DIAGNOSTIC TOOLS

Delayed intradermal skin testing (IDT) in SCAR was long considered a high-risk procedure. Indeed, certain reports describe disease reactivation following IDT, particularly in the first 4 to 6 weeks after the acute reaction. However, recent cohort studies have demonstrated this procedure's safety and clinical importance for the non-SJS/TEN phenotypes. Delayed IDT guides the

use of class-related antibiotics and implicated drugs following negative testing. The sensitivity of delayed IDT to antimicrobials ranges from 40% to 56% for severe phenotypes, excluding SJS/TEN. For patch testing, the sensitivity is highest for acute generalized exanthematous pustulosis (58%), DRESS (32%-64%), and maculopapular exanthema (10.8%-38.4%). However, the specificity and the false-positive rate remain unknown.

Further, in carefully selected phenotypes such as DRESS, oral drug challenge (ie, provocation) following skin testing is not associated with severe adverse drug reactions. These procedures are performed with the least-suspected or an alternative agent (eg, cephalosporin in penicillin-associated DRESS) following identification of the culprit agent by positive skin testing.¹ When drugs are needed for life-threatening infections (eg, tuberculosis, HIV), a direct rechallenge with the offending and/or structurally related drugs has been successfully deployed in SCAR. In the event of a mild-to-moderate adverse drug reaction, a corticosteroid-based "salvage" protocol has been successfully deployed.² This is still not without risk, as case reports demonstrate a phenotypic activation that can lead to fatality in severe cases such as SJS/TEN.³ Despite progress in the application of *in vivo* testing for SCAR, it remains in the domain of carefully selected patients and allergy centers.

IN VITRO/ EX VIVO DIAGNOSTIC TOOLS

In vivo testing is limited by the timing of the procedure, as no investigations can be offered in the acute setting. Some recommendations suggest waiting up to 6 months following skin disease resolution. In this scenario, *in vitro/ex vivo* tools are appealing, as the procedure's safety can be assured. Some specialized centers have developed laboratory tools to evaluate drug causality.^{4,5} The lymphocyte transformation test measures the average proliferation of drug-exposed blood based on the proliferation of negative control cultures and use of a radioactive thymidine (H-thymidine) uptake (Fig 1). Following stimulation with the culprit drug, the enzyme-linked ImmunoSpot (ELISpot) assay detects cytokines produced by CD4⁺ or CD8⁺ T cells in the patient's PBMCs or blister fluid cells.⁶ The accuracy of these tools seems to increase when studying either a specific drug or a disease phenotype. For lymphocyte transformation tests, the initially reported sensitivity of 27%⁷ increased to 73% in a cohort of 41 patients with DRESS.⁸ The specificity of this tool ranges from 82% to 100%. Most studies looking at the enzyme-linked ImmunoSpot assay include antibiotics as culprit drugs, with a reported sensitivity of 42% to 86% (specificity 100%) and vancomycin DRESS being a particularly well-described phenotype (Table I). The sensitivity for nonantibiotics is 63% to 100%, and the specificity is 95% to 100%. As for the phenotypes, a significant benefit has been shown in severe maculopapular exanthema

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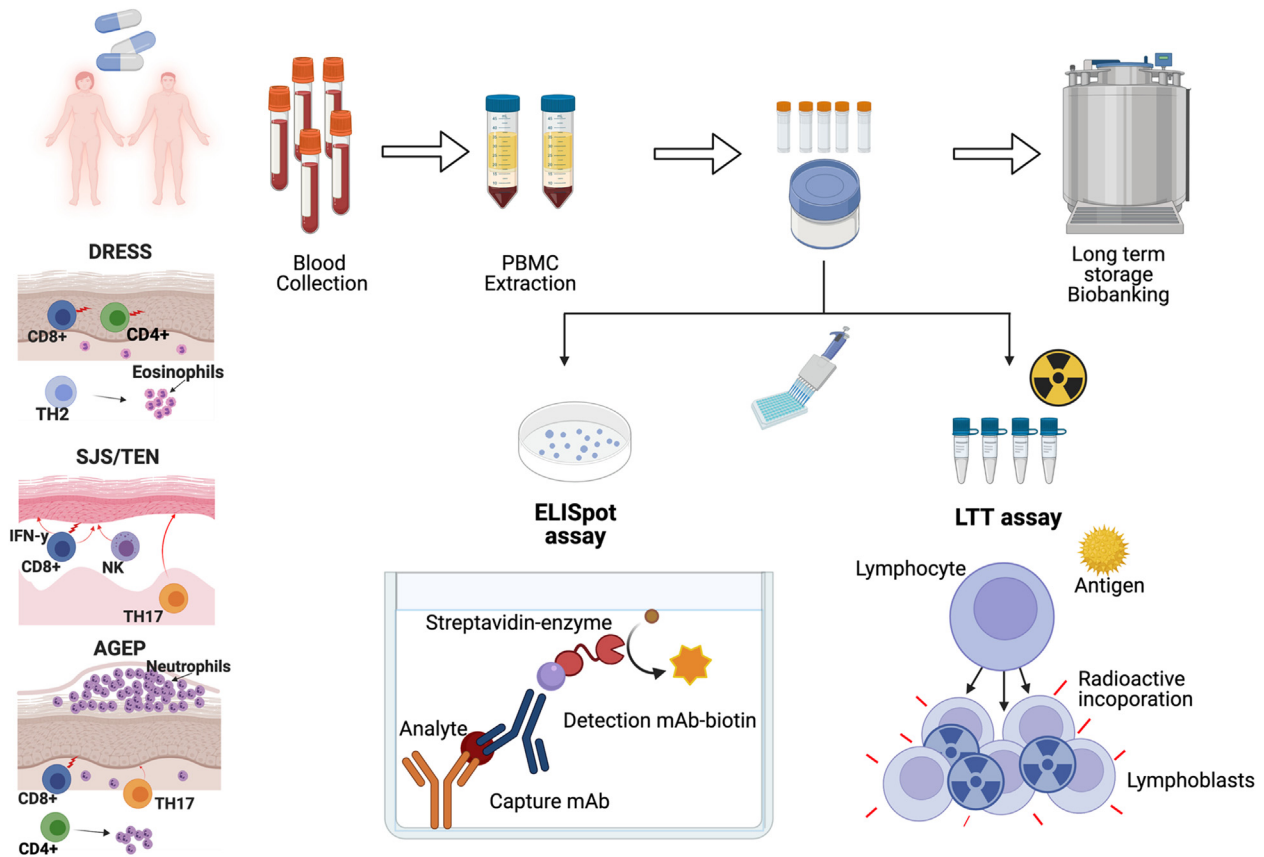


FIG 1. Blood collection and PBMC extraction, storage, and use in *ex vivo* assays for patients with DRESS, SJS/TEN, or acute generalized exanthematous pustulosis (AGEP). *Ex vivo* testing, such as the lymphocyte transformation test (LTT) and enzyme-linked ImmunoSpot (ELISpot) assay, from “bedside” to “bench” is illustrated. Indeed, for a patient diagnosed with severe cutaneous adverse reactions such as DRESS, SJS/TEN, or AGEP, a blood collection is required. PBMCs are extracted from the sample in the immunology laboratory. These PBMCs can be stored for future use or used immediately for 1 of the *ex vivo* tools. The LTT measures the average proliferation of drug-exposed blood based on the proliferation of negative control cultures and use of radioactive thymidine (H-thymidine) uptake. The ELISpot assay detects cytokines, presumed to be produced by CD4⁺ or CD8⁺ T cells, in the patient’s PBMCs or blister fluid cells following stimulation with the culprit drug. NK, Natural killer.

and DRESS, with greater evidence needed for the SJS/TEN phenotypes.

Nevertheless, the *in vivo* and *ex vivo* tools lack international validation and are unavailable in most health services. Indeed, the current *in vivo* testing methods (skin) and laboratory assays rely on drugs and/or drug metabolites, which are not effectively recognized by the immune system *ex vivo*.⁹ This can contribute to reduction of the test sensitivity. Returning to the “bench” to understand the key metabolites can be achieved only by understanding these epitopes’ immune recognition and binding. Using our understanding of HLA predictions with abacavir (HLA-B*57:01), vancomycin (HLA-A*32:01),¹⁰ and carbonic anhydrase inhibitors (HLA-B*59:01), for example, provides a valuable road map for the identification of novel pharmacogenomic predictors. Translation of these pharmacogenomic discoveries to clinical practice remains the key. Although successful translation has been seen with universal HLA-B*57:01 screening for abacavir hypersensitivity (number needed to test [NNT] to prevent 1 case = 13), the NNT (eg, HLA-B57*01 and flucloxacillin drug-induced liver injury; NNT = 13,819) and population carriage rate (eg, HLA-B58*01 and allopurinol SCAR) have been limiting in some cases.

As the sensitivity of both *in vivo* and *ex vivo* tools individually remains low (40%-73%), the concept of combination testing has been explored. In our experience, the combination of *in vivo* and *ex vivo* testing provided diagnostic results in 63% of our patients, identifying an additional 15 cases (19%) that could have been missed with skin testing alone.⁵ In the scenario of vancomycin DRESS, associations between DRESS and carriage of HLA class I and II alleles determined that HLA-A*32:01 is associated with vancomycin-induced DRESS in a population of European ancestry.¹⁰ To enhance the diagnostic approach for severe phenotypes, part of the solution could be to use traditional *in vivo* testing in combination with novel research-based *ex vivo* testing and pharmacogenomic risk prediction. Further, some life-threatening reactions might benefit solely from *ex vivo* investigations. Advancing these tools requires international collaboration, policy and guideline support, and standardization of *in vivo* testing protocols and concentrations. The diagnostic approaches will be enhanced by understanding the HLA predictors of SCAR stratified for ethnicity and varied populations. A significant evolution of these critical concepts will lead to the translation of *ex vivo/in vitro* assays into prospective

TABLE I. Sensitivity and specificity of the ELISpot and LTT assay based on the reported phenotype

Phenotype	Drug(s)	Sensitivity	Specificity
ELISpot assay			
DRESS	Vancomycin	61%	97%
	β-Lactams		
	Antiepileptics		
	Antituberculosis drugs		
	Allopurinol		
MPE	Sulfonamides	50%	90%
	β-Lactams		
SJS/TEN	Antiepileptics	49%	98%
	Allopurinol		
	Sulfonamides		
AGEP	β-Lactams	43%	86%
LTT assay			
DRESS	β-Lactams	62%	91%
	Antiepileptics		
	Antituberculosis drugs		
	Antifungals		
	Allopurinol		
MPE	Antituberculosis drugs	25%-58%	93%-100%
	Antiepileptics		
SJS/TEN	Antibiotics	27%	95%
	Antiepileptics		
AGEP	Beta-lactams	n/a	n/a
	Antibiotics		

Several of the studies for LTTs included antibiotics and nonantibiotic drugs. Sensitivity is the probability that an individual with the disease will test positive; a test with high sensitivity will not miss many patients with the disease. Specificity is the probability that an individual without the disease will test negative; a test with high specificity will infrequently identify patients as having a disease when they do not.

AGEP, Acute generalized exanthematous pustulosis; ELISpot, enzyme-linked ImmunoSpot; LTT, lymphocyte transformation test; MPE, maculopapular exanthema; n/a, not available.

international network trials and advancing a step closer to the allergist's toolkit.

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