Original Article

The Combined Utility of *Ex vivo* IFN-γ Release Enzyme-Linked ImmunoSpot Assay and *In vivo* Skin Testing in Patients With Antibiotic-Associated Severe Cutaneous Adverse Reactions

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What is already known about this topic? The individual use of *in vivo* skin testing and *ex vivo* IFN-γ release enzyme-linked immunoSpot (ELISpot) assay for assigning drug causality in severe cutaneous adverse reactions (SCARs) shows promise, yet the joint utility in antibiotic-associated SCARs remains ill-defined.

What does this article add to our knowledge? The combined use of *in vivo* and *ex vivo* diagnostics in antibiotic-associated SCARs assigned causality safely in 79% of cases, and IFN-γ release ELISpot assay demonstrated good sensitivity and high specificity.

How does this study impact current management guidelines? Skin testing (*in vivo*) and IFN-γ release ELISpot assay (*ex vivo*) are complementary approaches that may prove safe and effective in ascertaining antibiotic causality and improve, often difficult antibiotic prescribing, after SCARs.

BACKGROUND: For severe cutaneous adverse reactions (SCARs) associated with multiple antibiotics dosed concurrently, clinical causality is challenging and diagnostic approaches are limited, leading to constricted future antibiotic choices.
OBJECTIVE: To examine the combined utility of *in vivo* and *ex vivo* diagnostic approaches at assigning drug causality in a cohort of patients with antibiotic-associated (AA)-SCARs.
METHODS: Patients with AA-SCARs were prospectively recruited between April 2015 and February 2017. *In vivo* testing (patch testing or delayed intradermal testing) was performed to the implicated antibiotic(s) at the highest nonirritating concentration and read at 24 hours through 1 week. *Ex vivo* testing used patient peripheral blood mononuclear cells (PBMCs) stimulated with a range of pharmacologically relevant concentrations of implicated antibiotics to measure dose-dependent IFN-γ release from CD4+ and CD8+ T cells via an enzyme-linked immunoSpot assay.
RESULTS: In 19 patients with AA-SCARs, combined *in vivo* and *ex vivo* testing assigned antibiotic causality in 15 (79%) patients. Ten patients (53%) with AA-SCARs were positive on IFN-γ release enzyme-linked immunoSpot assay, with an overall reported sensitivity of 52% (95% CI, 29-76) and specificity of

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Abbreviations used
AA- Antibiotic-associated
AGEP- Acute generalized exanthematous pustulosis
DRESS- Drug reaction with eosinophilia and systemic symptoms
ELISpot- Enzyme-linked immunospot
IDT- Intradermal testing
IQR- Interquartile range
PT- Patch testing
SCAR- Severe cutaneous adverse reaction
SFU- Spot-forming unit
SJS- Stevens-Johnson syndrome
TEN- Toxic epidermal necrolysis

100% (95% CI, 79-100), with improved sensitivity noted in acute (within 1 day to 6 weeks after SCAR onset) testing (75%) and in patients with higher phenotypic scores (59%). There was increased use of narrow-spectrum beta-lactams and antibiotics from within the implicated class following testing in patients with a positive *ex vivo* or *in vivo* test result. CONCLUSIONS: We demonstrate the potential utility of combined *in vivo* and *ex vivo* testing in patients with AA-SCARs to assign drug causality with high specificity. **(J Allergy Clin Immunol Pract 2017;■:■-■)**

Key words: *Antibiotic allergy; Delayed hypersensitivity; Stevens-Johnson syndrome; toxic epidermal necrolysis; drug reaction with eosinophilia and systemic symptoms*

Severe cutaneous adverse reactions (SCARs), such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS), are associated with significant mortality and short-term and long-term morbidity[1,2] and may be caused by a range of medications including antibiotics.[1] SJS and TEN are considered the same condition representing different severities across a spectrum. The hallmarks of SJS/TEN are skin detachment (1%-10% for SJS, 10%-30% for SJS/TEN overlap, and >30% for TEN) and blistering of mucous membranes accompanied by other serious manifestations of systemic involvement.[3] Patients experiencing DRESS exhibit an exanthematous rash, fever, internal organ involvement, and possible eosinophilia.[3] Acute generalized exanthematous pustulosis (AGEP), another SCAR, is an acute widespread erythematous reaction that is followed by a pustular eruption together with fever.[3] To avoid the recurrence of SCARs, culprit drugs are traditionally avoided in the future.

Often in SCARs multiple antibiotics are prescribed concurrently, creating uncertainty in ascribing causality, which can lead to significant constriction of future therapeutic choices.[1,2] Current diagnostic options such as *in vivo* patch testing (PT) or delayed intradermal skin testing (IDT) have been limited by lack of experience, lack of validated concentrations and approaches, limited availability to providers, and poor sensitivity.[4-6] *Ex vivo* and *in vitro* testing using a range of research platforms including lymphocyte transformation testing and IFN-γ release enzyme-linked immunoSpot (ELISpot) assay have been used in small cohorts of antibiotic-associated (AA) delayed hypersensitivities with varied success.[7-12] Furthermore, there is scarce published literature on the utility of combination *in vivo* and *ex vivo/in vitro* approaches such as PT and/or delayed IDT with IFN-γ release ELISpot assay or lymphocyte transformation testing in AA-SCARs. The objectives of this pilot study were to examine the potential combined utility of IFN-γ release ELISpot assay and *in vivo* skin testing in defining antibiotic causality assessments in patients with AA-SCARs.

METHODS

Patient recruitment and definitions

Study patients were prospectively recruited at Austin Health, Alfred Health and Peter MacCallum Cancer Centre from April 2015 until February 2017. Inclusion criteria were patients 18 years or older with a history of AA-SCARs. Patients with AA-SCARs with an antibiotic identified as the primary implicated drug(s) and corresponding Naranjo adverse drug reaction score of 5 or more (probable adverse drug reaction)[13] were recruited. For the SJS/TEN phenotypes, an ALDEN score of 4 or more was required (as per published definitions[14]), with an antibiotic having to carry the highest ALDEN (algorithm for assessment of drug causality for epidermal necrolysis) score. For phenotypes of DRESS and AGEP, a RegiSCAR score of 2 or more and an AGEP score of 2 or more, respectively, were required.[15,16] All cases had the diagnosis and phenotype confirmed by a dermatologist and were reviewed in the respective hospital antibiotic allergy clinics (Austin Health and Peter MacCallum Cancer Centre). Patients with an alternative viral, bacterial, or autoimmune SCAR etiology were excluded, evidenced by any one of the following: (1) positive plasma PCR for herpesvirus (HSV1/2, cytomegalovirus, EBV) or Enterovirus, (2) positive *Mycoplasma* species PCR (respiratory specimen) or serology, or (3) detectable antinuclear antigen antibody titer of more than 1:64. Patients were also excluded if skin biopsy (histopathology or direct immunofluorescence) was not consistent with a drug reaction or clinical picture was consistent with an alternative diagnosis. There were 2 control groups: (1) antibiotic-tolerant controls, patients who had tolerated at least 4 consecutive weeks of single antibiotic at therapeutic intravenous or oral dosing, and (2) healthy random donors, patients with AA-SCARs tested against antibiotics that previously resulted in a positive IFN-γ release ELISpot assay.

Peripheral blood mononuclear cell (PBMCs) were isolated from whole heparinized blood of patients with AA-SCARs, tolerant controls, and healthy donors, washed, and counted. PBMCs were stored at −80°C in 90% heat-inactivated FBS and 10% dimethyl sulfoxide until use for IFN-γ release ELISpot assay. Patients were followed for adverse events and antibiotic prescribing for 90 days after testing. Ingestion challenge was not performed as routine after *ex* vivo and *in vivo* testing; rather, it was based on acute antibiotic requirements. This study was approved by the Austin Health Ethics Committee (HREC/15/Austin/75) and laboratories where this testing was performed had independent review board approvals (Institute for Immunology & Infectious Diseases, Murdoch [Murdoch University HREC 2011/056] University and Vanderbilt University Medical Center).

Skin testing (*in vivo*)

IDT and PT were performed for all implicated antibiotics at least 6 weeks after AA-SCAR onset using previously recommended nonirritating antibiotic concentrations.[17-19] In patients in whom an intravenous formulation of the implicated antibiotic was not available or incompatible with IDT and/or in the setting of SJS/TEN, PT was performed in isolation. IDT was performed on the volar forearm of the skin with 0.02 mL of antibiotic reagent or normal