Human Leukocyte Antigens as Determinants of Drug-Specific Immune Responses and Genetic Risk Factors for SCARs

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Abstract

Background: Research efforts have been concentrated to develop new biomarkers with a particular interest in cytokines and chemokines released from activated T cells. Studies on cytokine measurements after clinical drug challenge in SCARS patients have reported an initial increase in serum TNF-α and IL-8 followed by elevation in IFN-γ, IL-6 and IL-10 levels. Early studies suggesting a weak association between some HLA serotypes and SJS/TEN. But later on a strong connection between HLA alleles and drug-induced cutaneous reactions has been identified in numerous studies. It is of note that the HLA encoding genes are the most polymorphic of the whole human genome, and the distribution of the various alleles is quite heterogeneous across human populations from different geographic locations. This makes necessary specific studies on defined ethnic groups and hinders the identification of strongly associated HLA alleles in populations with high rates of genetic exchange resulting from migration, such as the populations of Europe and North America. In this regard, it is interesting that the prognostic value of HLA-B*57:01 as a risk factor to develop abacavir hypersensitivity was initially questioned after reports of low sensitivity in some population groups such as Hispanic patients and those of African descent. However, further studies showed a strong association for patch test-confirmed cases across all ethnicities.

Keywords: Human Leukocyte Antigens, Drug-Specific Immune Responses

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Introduction:

Research efforts have been concentrated to develop new biomarkers with a particular interest in cytokines and chemokines released from activated T cells.

Studies on cytokine measurements after clinical drug challenge in SCARS patients have reported an initial increase in serum TNF-α and IL-8 followed by elevation in IFN-γ, IL-6 and IL-10 levels **(Shiohara et al., 2015).** Similarly, dosage of levels for multiple cytokines/chemokines in order to identify essential markers has also been attempted with studies identifying a significant increase in IL-6 and interferon gamma-produced protein 10 (IP-10) in SJS/TEN and DRESS as well as IL-16 in FDE, SJS and DRESS but not TEN **(Shiohara et al., 2015).**

These authors go to recommend the use of IL-6 and IL-10 as diagnostic and predictive tools in monitoring adverse drug reactions **(Shiohara et al., 2015).** But these markers may be elevated in other conditions such as acute infection and sepsis. Further, serum soluble Fas-ligand (sFasL) levels, granulysin, IL-15, CD137 and the proapoptotic factor galectin-7 have been described in the pathological processes of SJS/TEN with sFasL and galectin-7 being considered as biomarkers able to predict TEN progression but not SJS and granulysin serum levels correlating with disease severity and mortality **(Hama et al., 2019).**

In DRESS/DiHS, several markers were reported as indicators of disease progression and activity such as the serum thymus and activation-regulated chemokine (TARC) and granulysin. Other markers such IL-2, IL-4, IL-5, IL-13, IFN-γ and granzyme-B have been described in T-cell drug hypersensitivity **(Komatsu-Fujii et al., 2018).** Measurement of these markers was reported using the ELISpot, intracellular cytokine staining, ELISA, rapid immunochromatographic tests, plex bead-based immunoassay kits and flow cytometry**(Su et al., 2016).**

Controversial markers are also important to underline such as IL-17 with some studies reporting a negative correlation with adverse drug reactions **(Shiohara et al., 2015)** while others described an increase of this cytokine in SJS/TEN. Similarly, procalcitonin has been described as a marker for bacterial infection that could benefit the differential diagnostic that includes delayed hypersensitivity **(Yoon et al., 2013).**

Early studies suggesting a weak association between some HLA serotypes and SJS/TEN. But later on a strong connection between HLA alleles and drug-induced cutaneous reactions has been identified in numerous studies **(Su SC etal 2016).** These seminal investigations have been followed by HLA-typing studies confirming the significant associations of these alleles across various populations in which the respective alleles are prevalent (**White etal 2018**), and (**Chen etal 2018**).

It is of note that the HLA encoding genes are the most polymorphic of the whole human genome, and the distribution of the various alleles is quite heterogeneous across human populations from different geographic locations. This makes necessary specific studies on defined ethnic groups and hinders the identification of strongly associated HLA alleles in populations with high rates of

genetic exchange resulting from migration, such as the populations of Europe and North America **(Phillips 2019).**

In this regard, it is interesting that the prognostic value of HLA-B*57:01 as a risk factor to develop abacavir hypersensitivity was initially questioned after reports of low sensitivity in some population groups such as Hispanic patients and those of African descent. However, further studies showed a strong association for patch test-confirmed cases across all ethnicities **(Sousa-Pinto etal 2016).**

Previous studies found an association between HLA-B*15:02and HLA-A*31:01 and carbamazepine-induced SJS/TEN (**Genin etal 2014).** Interestingly, the association between HLA-A*31:01 and carbamazepine-induced MPE had been previously described in Han Chinese patients Associations between HLA-B*38:01 and lamotrigine-induced SJS/TEN and between HLA-A*24:02 and lamotrigine-induced DRESS have been reported in Spanish patients (**Ramírez etal 2017**). The association between HLA-B*38:01 and lamotrigine-induced SJS/TEN had been previously reported among European patients **(Lonjou etal 2008).** On the other hand, HLA-A*24:02 was found to be a genetic risk factor for lamotrigine-induced MPE in Norwegian **(Shirzadi etal 2015)** and Korean populations **(Moon etal 2015).**

Strong associations were recently identified between HLA-B*13:01 and dapsone-induced hypersensitivity reactions in Asians **(Zhang etal 2013),** between HLA-B*59:01 and methazolamide-induced SJS/TEN in Korean, Japanese and Han Chinese patients and between HLA-A*32:01 and vancomycin-induced DRESS in North American patients (**Konvinse etal 2019).**

Table 1: Human leukocyte antigen class I alleles with well-established associations in severe cutaneous adverse reactions (**Tangamornsuksan and Lohitnavy 2018).**

HLA risk allele	Drug	SCAR	Population	Population OR	
HLA-B*57:01	Abacavir HSS		Abacavir hypersensitivity	All	960
HLA-B*58:01	Allopurinol	SJS/TEN	DRESS	All	580
HLA-B*15:02	Carbamazepine	SJS/TEN		South East Asian	> 1000
HLA-A*31:01	Carbamazepine		DRESS	European, South East Asian, Japanese	57.6
HLA-B*13:01	Dapsone		DRESS	All	20
HLA-B*59:01	Methazolamide	SJS/TEN		Han Chinese, Korean, Japanese	715.3

HLA-A*32:01	Vancomycin		DRESS	North American (European ancestry)	70

The identification of certain HLA alleles as risk factors has impelled the implementation of genetic testing for the prevention of severe hypersensitivity reactions **(Jung etal 2015).** HLA-B*57:01 has 100% negative predictive value (NPV) for abacavir hypersensitivity reactions and 55% positive predictive value (PPV) **(White etal 2018).** This makes HLA-B*57:01 testing highly cost effective for the prevention of hypersensitivity reactions. HLA-B*57:01 screening before abacavir prescription is part of guideline-based routine HIV practice in the developed world.

With respect to other HLA alleles that show strong associations with SCARs, high NPVs have also been calculated for HLA-B*15:02 in relationship with carbamazepine-induced SJS/TEN and for HLA-B*58:01 in allopurinol-induced SCARs in Asian populations. Although the PPV is low in both cases **(White etal 2018)**, HLA-B*15:02 screening before carbamazepine prescription has been introduced into routine clinical practice in several Southeast Asian countries such as Taiwan, Singapore, and Hong Kong, where significant reductions in carbamazepine-associated SJS/TEN have been achieved **(Chen etal 2018).**

Diagnostic Methods of SCARS

❖ **In vivo testing:**

1. Drug Challenge:

In the context of drug allergy, drug challenge in a patient with suspected drug-induced hypersensitivity remains the gold standard for determining tolerance **(Aberer et al., 2003).** For immediate reactions, such as IgE mediated reactions, a negative drug challenge has a 100% negative predictive value. However, in the case of a severe delayed reaction, re-challenge with a single dose of a drug may not reproduce the reaction and, hence, it has a lower sensitivity than a prolonged challenge (3–5 days), particularly with a remote reaction **(Hjortlund et al., 2012). (Trubiano et al., 2017a**).

In addition, with high severity reactions, drug challenge carries an inherent risk and the benefit of re-challenge has to be carefully weighed against the risk of a serious reaction. In cases of SCARS or severe organ involvement, challenges are contraindicated because of the risk of a life-threatening clinical reaction **(Rive et al., 2013).**

In this context, investigational tools have been developed to aid drug evaluation. In vivo testing such as Patch test (PT) and delayed intradermal test (IDT) and ex vivo assays such as the LTT and ELISpot have been described for various drugs and phenotypes but lack international validation. Combining in vivo and ex vivo methods in delayed hypersensitivity reactions can increase the

diagnostic yield, although this has been shown in only small cohort studies **(Trubiano et al., 2018).**

2. Skin Testing:

In vivo testing (PT and delayed IDT) is usually performed to the implicated drug(s) at least 4–6 weeks after delayed hypersensitivity resolution at the recommended non-irritating concentrations **(Phillips et al., 2019).**

2.1 Patch Testing:

The main types of reactions where PT is used with specificity are MPE, AGEP, DRESS, SJS/TEN and FDE **(Barbaud et al., 2013).** The sensitivity of this investigational tool varies depending on the clinical setting, the causal drug, the drug concentrations used and the phenotype with typical figures for AGEP at 58–64%, DRESS between 32 and 80%**)** and SJS/TEN, 9–24% **(Barbaud et al., 2013).** Drugs like antiepileptics, contrast media, beta-lactams, tetrazepam and pristinamycin increase the sensitivity of PT, while allopurinol or its active metabolite, oxypurinol, appear to never provide clinical utility **(Johansen et al., 2015**).

The testing should be performed at least one month after the resolution of the reaction or after discontinuation of oral steroids, as immunosuppressants can decrease T-cell mediated immunity, and preferably during the first year after the reaction. The European Network on drug allergy (ENDA) and the European Academy of Allergy and Clinical Immunology (EAACI) recommend timing between 3 weeks and 3 months and describe drug concentrations between 5 and 30% with most antimicrobials diluted at 20**%** or 30% in petrolatum vehicle and the retained vehicle alone as negative control **(** (**Brockow et al., 2019).**

For DRESS, patch testing may be further delayed because of the concomitant dosing of topical or systemic steroids or other immunosuppressants and to avoid confusion with DRESS relapse. Available literature suggests that the yield from patch testing for SJS/TEN is in general low but dependent on the drug and class of drugs. Sensitivities will vary from 0% for allopurinol to >50% for aromatic antiepileptic drugs such as carbamazepine **(Konvinse et al., 2016).**

The two forms of PT described are the extemporaneous, involving the local preparation of the PT by the pharmacy or the drug allergy staff with commercially available drugs and petrolatum or water, and the conventional PT implying use of a limited number of ready-to-use commercialized PT products at 10% concentration in petrolatum. In a retrospective study, 21/75 (23.3%) patients with MPE, FDE, AGEP, DRESS, SJS/TEN tested simultaneously with both methods had positive results, indicating that both methods are as valuable and reliable **(Assier et al., 2017).**

PT is usually applied in the upper back regions for practical reasons with the exception of FDE in which the PT is applied on the region of the previous reaction. The International Contact Dermatitis Research Group have published an interpretation score for the patch test reactions **(Barbaud et al., 2014).**

In a large multi-center patch testing cohort, only one patient (1/134) presented a relapse of his skin condition (AGEP) following patch testing **(Barbaud et al., 2013)** indicating that this diagnostic method carries low morbidity. In a retrospective review including 826 patients, PT showed promising results for drug challenge outcomes with 82.3% (14/17) with positive PT having a positive challenge and 90.4% (207/229) patients with negative PT presenting no reaction to challenge **(Barbaud et al., 2013).**

PT is a quick and safe investigational method clinically relevant when testing is conclusive, a negative PT not excluding the possibility that the drug is causal. There is need to re-challenge negative testing in less severe clinical phenotypes. This method should be homogenized, as to resolve current inconsistencies, by comparing the outcomes in large multicenter studies, determining concentration thresholds and avoiding false negative and false positive results **(Konvinse et al., 2016).**

2.2 Intradermal Testing:

Intradermal testing is done on the volar aspect of the forearm with 0.02–0.05 ml of antibiotic reagent or normal 0.9% serum saline (negative control) **(Brockow et al., 2019).** The use of IDT is limited to drugs available in liquid sterile formulations. The positive control normally used is a skin prick test with histamine 10 mg/ml **(Heinzerling et al., 2013).** In terms of drug concentrations, expert consensus advises the use of the highest non-irritating concentration described for immediate reactions **(Phillips et al., 2019).**

However, recent work for drugs with non-IgE mast cell activation determined that higher concentrations that might initially be irritating are needed for improved sensitivity (i.e., ciprofloxacin, vancomycin) **(Konvinse et al., 2016).** An IDT result is considered positive when the dermal induration and erythema at the injection site exceeded 5 mm from baseline. Delayed reading is performed at 24, 48 h and up to 1 week **(Brockow et al., 2019).** IDT with delayed reading has been described in reactions such as MPE, AGEP and DRESS with potential risk in SJS/TEN and unknown utility in FDE. This investigational tool was previously considered potentially harmful in SCAR phenotypes but actually few reports describe severe systemic reactions following IDT For SJS/TEN, based on the current available literature, the benefit of IDT does not outweigh the risk. For DRESS, it is recommended that testing generally be done 6 months following the acute reaction **(Syrigou et al., 2016; Watts, 2017).**

In terms of cross-reactivity between beta-lactams in the context of delayed hypersensitivities, 18.7–31.2% of the patients tested presented a reaction to amino-penicillins and amino-cephalosporins predicted by the presence of shared R1 and R2 side chains **(Romano et al., 2016).** Also, in patients with a delayed penicillin type reaction, delayed IDT to beta-lactams has allowed to confirm tolerance to cephalosporins **(Picard et al., 2019; Trubiano et al., 2020),** carbapenems **(Picard et al., 2019)** and monobactams**.** Other classes of interest are currently being studies with no evidence of cross-reactivity such as glycopeptides, antibiotic and non-antibiotic sulfonamides, drugs in the rifampin class and aromatic and non-aromatic anticonvulsants **(Heinzerling et al., 2013).**

In the setting of a severe delayed reaction, PT is related to lower adverse reactions but IDT has been described as more sensitive in non-SJS/TEN reactions while some recommendations only suggest proceeding to IDT after negative PT. In a cohort study of 21 patients with delayed reactions to penicillin and 30 controls with no allergic history, no false positives were reported and 20/21were positive for IDT compared to 18/21 for patch testing**.** Widespread implementation of IDT for delayed hypersensitivities still carries some barriers such as the lack of available sterile preparation for all drugs, generally low negative predictive value (NPV) and limited data in some reactions**(Cabanas et al., 2014).**

❖ **Ex Vivo Diagnostic Tools:**

In vitro/ex vivo diagnostics, such as the LTT and the ELISpot assay, while having the advantage of carrying no risk of drug re-exposure for the patient, are not available for routine diagnostic use in most centers.

1. Lymphocyte Transformation Test:

LTT has been extensively studied as a diagnostic method for delayed hypersensitivity reactions. Lymphocytes are isolated from the patient's peripheral blood mononuclear cells (PBMC) and cultured with pharmacological concentrations of the suspected drugs for 5–7 days. LTT responses are measured by the stimulation index (SI, average proliferation of drug-exposed cultures/average proliferation of negative control cultures), with typically an SI > 2+ confirming response, which is calculated based on the radioactive thymidine (H-thymidine) uptake, a marker directly proportional to the degree of T-cell proliferation in response to a drug antigen **(Kato etal 2017).**

This enhanced response is interpreted as a T-cell sensitization and has produced positive responses in different clinical settings and with various implicated drugs (**Rive et al., 2013).** However, one might keep in mind that lymphocyte stimulation can occur not only by immunological mechanisms but also pharmacological ones and some drugs may cause false positive results as was observed in some patients that presented positive responses to drugs they had tolerated **(Kato etal 2017).**

The reported sensitivity of LTT in delayed hypersensitivity reactions ranges from 27% to 74 and specificity was quoted as 85% to 100% **(Porebski et al., 2013; Porebski et al., 2015).** Putting aside the demanding and time-consuming laboratory manipulations and the use of radioactivity and specialist equipment, the LTT can be an interesting support in drug hypersensitivity diagnosis but is still only used as a research tool **(Kato etal 2017).** In the last 10 years, aside from case reports or small cases series**,** very few studies have focused solely on the LTT method for diagnosis **(Tomida et al., 2016).**

3. Enzyme Linked ImmunoSpot Assay:

Interferon gamma (IFN-γ, also known as Type II interferon) is an important immunoregulatory cytokine that was originally identified through its anti-viral activity. It plays key roles in host defense by exerting anti-viral, anti-proliferative and immunoregulatory activities. IFN-γ induces the production of cytokines and upregulates the expression of various membrane proteins including class I and II MHC antigens, Fc receptors, leukocyte adhesion molecules, and B7 antigen. IFN-γ is a potent activator of macrophage effector functions **(Copaescu et al. 2021).**

It potentiates the secretion of immunoglobulins by B cells and affects isotype switching. IFN-γ also influences T-helper cell phenotype determination by inhibiting Th2 differentiation and stabilizing Th1 cells . IFN-γ is produced primarily by activated NK cells, activated Th1 cells and activated CD8+ cytotoxic cells. Additional cell types that produce IFN-γ include macrophages, mast cells, dendritic cells, neutrophils , and peripheral γδ T cells. The production of IFN-γ is upregulated synergistically by IL-12 and IL-18. Human IFN-γ cDNA encodes a 166 amino acid (aa) residue precursor protein containing a 23 aa residue predicted signal peptide that is cleaved to generate the 143 aa residue mature human IFN-γ containing a pyroglutamate residue at the N-terminus **(Copaescu et al. 2021).**

Natural IFN-γ is heterogeneously glycosylated and contains truncations of up to 16 aa residues at the carboxy-terminus. In solution, human IFN-γ exists exclusively as a noncovalent homodimer. The functional IFN-γ receptor complex consists of two distinct subunits. The α subunit (IFN-γ R1) binds IFN-γ with high-affinity and species-specificity **(Velázquez et al, 2017).**

The β subunit (IFN-γ R2, also referred to as the accessory factor 1, AF-1) interacts with the IFN-γ occupied α subunit in a species-specific manner and is required for signal transduction via the JAK-STAT pathway. Both the α and the β subunits are type I membrane proteins. Whereas the α subunit is expressed constitutively at low levels on many cell types, the cellular expression of the β subunit correlates with IFN-γ responsiveness and is tightly-regulated. The Human IFN-γ ELISpot assay is designed for the detection of human IFN-γ secreting cells at the single cell level and can be used to quantitate the frequency of human IFN-γ secreting cells **(klaewsongkram etal 2019).**

As regards to ELISpot assays, they are well suited for monitoring immune responses to various stimuli treatments and therapies, and the have been used for the quantitation of antigen-specific

CD4+ and/or CD8+ T cell responses. Other methods for assessment of antigen-specific T cell responses, such as chromium release assays with quantitation by limiting dilution, are tedious and require previous in vitro expansion of T cells for several days. These assays typically are not suitable for measuring infrequent T cell responses that occur at less than 1 in 1000. ELISpot assays are highly reproducible and sensitive, and can be used to measure responses with frequencies well below 1 in 100,000. ELISpot assays do not require prior in vitro expansion of T cells and they are suitable for high-throughput analysis using only small volumes of primary cells **(Copaescu et al. 2021).**

PRINCIPLE OF THE ASSAY:

The ELISpot assay was originally developed for the detection of individual B cells secreting antigen-specific antibodies. This method has since been adapted for the detection of individual cells secreting specific cytokines or other antigens. ELISpot assays employ the quantitative sandwich enzyme-linked immunosorbent assay technique **(Trubiano et al., 2018).**

A monoclonal antibody specific for human IFN-γ has been pre-coated onto a polyvinylidene difluoride (PVDF)-backed microplate. Appropriately stimulated cells are pipetted into the wells and the microplate is placed into a humidified 37 °C CO2 incubator for a specified period of time. During this incubation period, the immobilized antibody in the immediate vicinity of the secreting cells binds secreted IFN-γ. After washing away any cells and unbound substances, a biotinylated polyclonal antibody specific for human IFN-γ is added to the wells. Following a wash to remove any unbound biotinylated antibody, alkaline-phosphatase conjugated to streptavidin is added **(Kato etal 2017).**

Unbound enzyme is subsequently removed by washing and a substrate solution is added. A blue-black colored precipitate forms and appears as spots at the sites of cytokine localization, with each individual spot representing an individual IFN-γ secreting cell. The spots can be counted with an automated ELISpot reader system or manually using a stereomicroscpe **(Kato etal 2017).**

Quantification of IFN-gamma expression by T cells is a well established surrogate test for assessing cellular-mediated immunemresponses. Several IFN-gamma detection methods have been developed and are commercially available, all characterized by specific discriminating features. In particular the enzyme-linked immunosorbentassay, enzyme-linked immunospot assay and intracellular cytokine staining assay are in widespread use. However, when measuring low-level responses, the ELISpot assay is a better choice due to its lower detection limit with high sensitivity. Moreover, it is less expensive to perform, less dependent upon sophisticated instrumentation and better suited to the analysis of frozen samples when limited numbers of cells are available **(klaewsongkram etal 2019).**

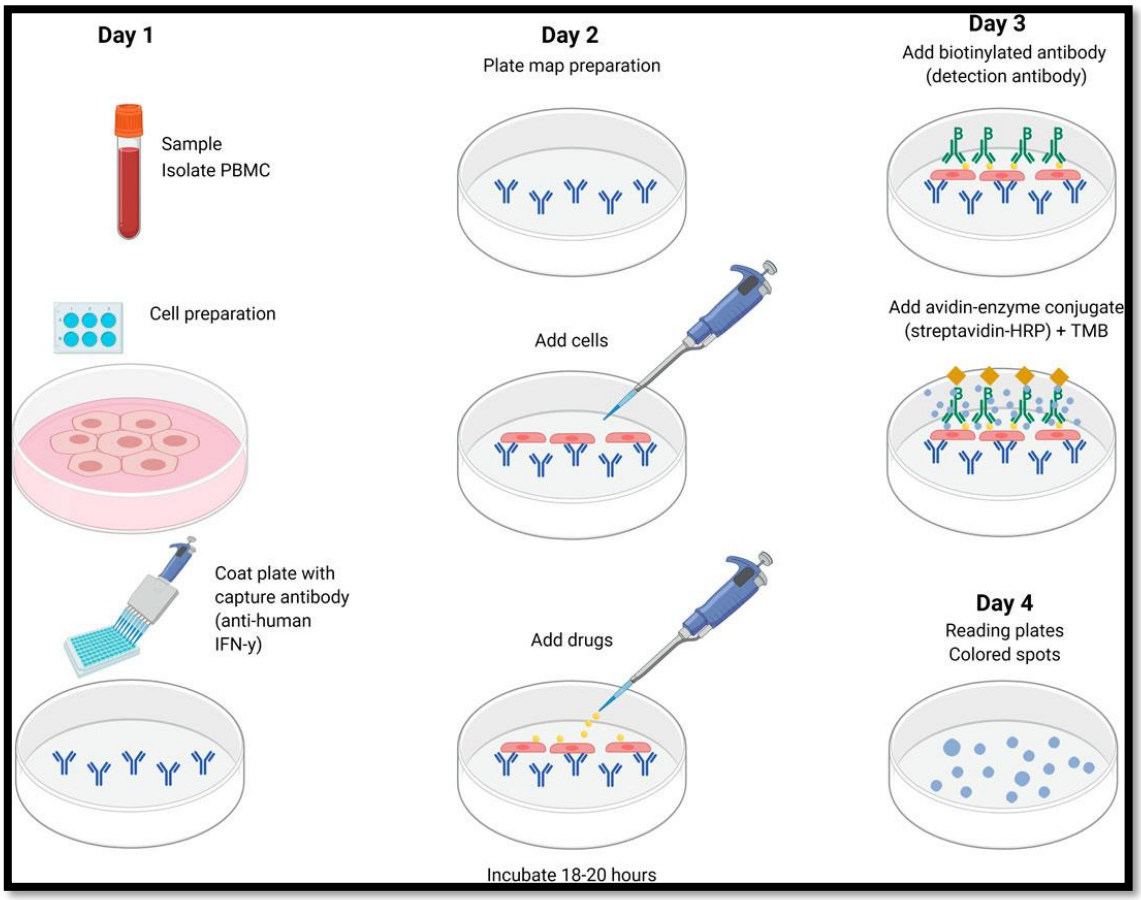

Figure 1: Detection of cytokine secretion using ELISpot **(Copaescu et al. 2021).**

Cytokine-specific coating antibody is added and incubated overnight at 4°C. The plate is washed and PBMCs and drug(s) are added and incubated for 18–20 h at 37°C. The following day, the plate is washed again and biotin-conjugated detection antibody is added and incubated for 2 h at room temperature. After another wash, streptavidin-bound enzyme is added and incubated for 1 h at room temperature. After the last wash, substrate is added (BCIP-NBT or TMB). Spot development is monitored for ~15 min (in dark). The plate is washed and left to dry overnight for a final reading on the fourth day. BCIP-NBT, 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT); ELISpot, enzyme linked ImmunoSpot; PBMC, peripheral blood mononuclear cells; TMB, tetramethyl benzidine.

Practical Approach to the Diagnosis of Cutaneous Adverse Drug Reactions

Dr. Shear suggested clinical assessment of drug-induced skin injury called the 4 Ds by: diagnosis of the adverse event, differential diagnosis, drug exposure, and determining probabilities **(Shear and Dodiuk-Gad 2019).**

1. Diagnosis of the Adverse Event:

Any patient taking medications with a cutaneous eruption should be evaluated for a cutaneous ADR to determine if the clinical signs are a result of the medication or related to a different cause. The diagnosis of a cutaneous ADR is based on three key steps. The first step is determining morphology, according to the four main categories: exanthematous, urticarial, pustular, and blistering. The second step is to determine if there is any systemic involvement in addition to the cutaneous involvement as this will help to distinguish between a "simple" and a "complex" reaction**(Shear and Dodiuk-Gad 2019).**

The third step is determining the histology. Drug eruptions elicit a variety of inflammatory disease patterns histologically and can be used to help differentiate between the various cutaneous adverse drug reactions. Hence, skin biopsies can be very useful in the process of diagnosing cutaneous adverse drug reactions **(Orime 2017).**

2. Differential Diagnosis:

It is essential to establish a differential diagnosis, keeping in mind all the possible diagnoses. In addition, it is encouraged to rank the approximate likelihood of each condition **(Dodiuk-Gad et al. 2017).**

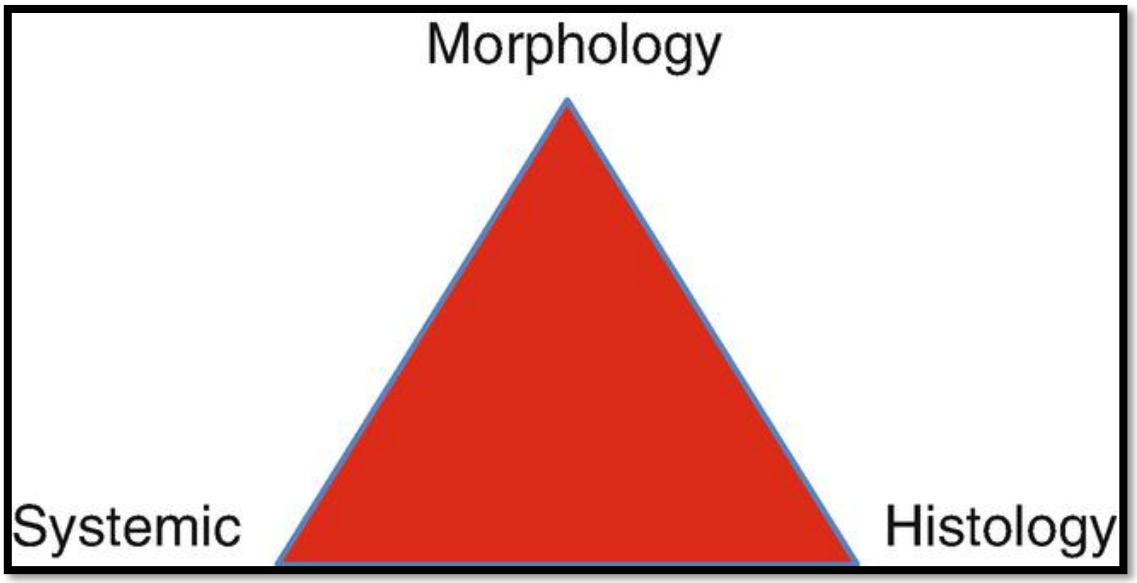

Figure 2. Dr. Shear's diagnostic triangle of reactions **(Shear and Dodiuk-Gad 2019).**

3. Drug Exposure (Drugs Taken and the Lag Period)

First and foremost, it is important to determine drug exposures. Physicians could consider obtaining the assistance of family members, pharmacists, and others that might know which medications the patient was taking prior to the adverse event. In addition, the lag period of a drug

reaction is crucial to analyze carefully when determining the causative agent since different cutaneous drug reactions have different timelines. The lag period is the time between initiation of the drug and the onset of the first symptoms of the drug reaction. All medications, especially those taken in the 8 weeks prior to the cutaneous adverse drug reaction, must be considered as possible causative agents. Physicians should also ask patients about over-the-counter medications in addition to prescription drugs **(Nigen et al. 2003).**

Physicians may want to consider creating a drug exposure timeline chart to visualize the chronology. The timeline could include relevant information including the first day of taking the medication, dosage, and the last day that the patient took the medicationmfor each drug as well as adding the signs and symptoms throughout this time period **(Dodiuk-Gad et al. 2017).** The time frame is very useful because it is influenced by the reaction pattern. For example, a drug eruption that is caused by an IgE-mediated mechanism will occur approximately minutes after a drug's initiation, while a reaction such as SJS/TEN will take several weeks to begin after a drug's initiation **(Young and Shear 2017).**

4. Determine Probabilities

The biggest challenge in assessing drug-induced skin injury is establishing whether it was caused by a medication a patient was taking or another cause that is unrelated. In order to effectively assess a causal relationship, it is crucial to take a thorough and detailed history including asking patients if they had any history of cutaneous rashes themselves or in their family and whether withdrawing the drug improved the eruption.

Physicians may also rate the reaction based on Naranjo et al.'s adverse drug reaction probability scale, which classifies the drug reaction according to a probability category as definite, probable, possible, or doubtful **(Dodiuk-Gad et al. 2017).**

Table 2: summarizes the major steps in the systematic approach for the diagnosis of cutaneous drug eruptions **(Shear and Dodiuk-Gad 2019).**

1	**Determination of the morphology of the primary lesion based on four main categories: exanthematous, urticarial, bullous, and pustular**
2	Assessment of systemic involvement based on patient's vital signs and symptoms and basic laboratory screen: full blood count, liver and renal function tests, and urine analysis
3	Skin biopsy for histopathology and if relevant direct immunofluorescence studies
4	The usage of validated diagnostic criteria, if available
5	Establishment of a working diagnosis and a differential diagnosis that takes into account all possible diagnoses
6	Drug exposure analysis—determination of the lag period and creation of a timeline
7	Determination of probabilities for the associations between the suspected drug and the clinical event according to patients' history, literature, and in vitro and in vivo diagnostic assessments including HLA genetic tests
8	Based on steps 6 and 7, identification of the suspected drugs
9	Communication with the patient, family, healthcare providers, and regulatory agencies
10	Proper follow-up for the patient in a timely and appropriate manner

References:

1. Aberer, W., Bircher, A., Romano, A., Blanca, M., Campi, P., Fernandez, J., et al. (2003). Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. Allergy. 58 (9), 854–863. doi:10.1034/j.1398-9995.2003.00279.x.
2. Agache I, Bilò M, Braunstahl GJ, Delgado L, Demoly P, Eigenmann P, Gevaert P, Gomes E, Hellings P, Horak F, Muraro A, Werfel T, Jutel M. In vivo diagnosis of allergic diseases--allergen provocation tests. Allergy. 2015 Apr;70(4):355-65. doi: 10.1111/all.12586. Epub 2015 Feb 12. PMID: 25640808.
3. Alfirevic A, Pirmohamed M (2017) Genomics of adverse drug reactions. Trends Pharmacol Sci 38(1):100–109
4. Ang CC, Wang YS, Yoosuff EL, Tay YK (2010) Retrospective analysis of drug-induced hypersensitivity syndrome: a study of 27 patients. J Am Acad Dermatol 63(2):219–227.
5. Anquetil C, Salem JE, Lebrun-Vignes B, Touhami S, Desbois AC, Maalouf G, Domont F, Allenbach Y, Cacoub P, Bodaghi B, Saadoun D (2020) Evolving spectrum of drug-induced uveitis at the era of immune checkpoint inhibitors results from the WHO's pharmacovigilance database. J Autoimmun 111:102454.

6. Assier, H., Valeyrie-Allanore, L., Gener, G., Verlinde Carvalh, M., Chosidow, O., and Wolkenstein, P. (2017). Patch testing in non-immediate cutaneous adverse drug reactions: value of extemporaneous patch tests. Contact Dermatitis. 77 (5), 297–302. doi:10.1111/cod.12842.
7. Barbaud A, Collet E, Milpied B, Assier H, Staumont D, Avenel-Audran M, Grange A, Amarger S, Girardin P, Guinnepain MT, Truchetet F, Lasek A, Waton J; Toxidermies group of the French Society of Dermatology. A multicentre study to determine the value and safety of drug patch tests for the three main classes of severe cutaneous adverse drug reactions. Br J Dermatol. 2013 Mar;168(3):555-62. doi: 10.1111/bjd.12125. PMID: 23136927.
8. Barbaud, A. (2014). Skin testing and patch testing in non-IgE-mediated drug allergy. Curr. Allergy Asthma Rep. 14 (6), 442. doi:10.1007/s11882-014-0442-8.
9. Barbaud, A., Collet, E., Milpied, B., Assier, H., Staumont, D., Avenel-Audran, M., et al. (2013). A multicentre study to determine the value and safety of drug patch tests for the three main classes of severe cutaneous adverse drug reactions. Br. J. Dermatol. 168 (3), 555–562. doi:10.1111/bjd.12125.
10. Bastuji-Garin S, Fouchard N, Bertocchi M, Roujeau JC, Revuz J, Wolkenstein P (2000) SCORTEN: a severity-of-illness score for toxic epidermal necrolysis. J Invest Dermatol 115(2):149–153. https:// doi. org/ 10. 1046/j. 1523- 1747. 2000. 00061.x.
11. Cabanas, R., Calderon, O., Ramirez, E., Fiandor, A., Prior, N., Caballero, T., et al. (2014). Piperacillin-induced DRESS: distinguishing features observed in a clinical and allergy study of 8 patients. J Investig. Allergol. Clin. Immunol. 24 (6), 425–430.
12. Cacoub P, Musette P, Descamps V, Meyer O, Speirs C, Finzi L, Roujeau JC (2011) The DRESS syndrome: a literature review.Am J Med 124(7):588–597. https:// doi. org/ 10. 1016/j. amjmed.2011. 01. 017.
13. Cardone M, Garcia K, Tilahun ME, Boyd LF, Gebreyohannes S, Yano M, et al. A transgenic mouse model for HLA-B*57:01– linked abacavir drug tolerance and reactivity. J Clin Investig. 2018;128(7):2819–32.
14. Cervera R, Rodriguez-Pinto I, Espinosa G (2018) The diagnosis and clinical management of the catastrophic antiphospholipid syndrome: a comprehensive review. J Autoimmun 92:1–11.
15. Chen CB, Wu MY, Ng CY, Lu CW, Wu J, Kao PH, Yang CK, Peng MT, Huang CY, Chang WC, Hui RC, Yang CH, Yang SF, Chung WH, Su SC (2018) Severe cutaneous adverse reactions induced by targeted anticancer therapies and immunotherapies. Cancer Manag Res 10:1259–1273.
16. Chen X, Oppenheim JJ. Th17 cells and T regs: unlikely allies.mLeukoc Biol. 2014;95(5):723–31.
17. Chung SJ, Ahn KM, Oh JH, Shim JS, Park HW (2020) Incidence rates of severe cutaneous adverse reactions due to antiseizure medication: a nationwide study using health claims data in Korea. Epilepsia. https:// doi. org/ 10. 1111/ epi. 16751.

18. Chung, W. H., Chang, W., Stocker, S. L., Juo, C. G., Graham, G. G., Lee, M. H., et al. (2015). Insights into the poor prognosis of allopurinol-induced severe cutaneous adverse reactions: the impact of renal insufficiency, high plasma levels of oxypurinol and granulysin. Ann. Rheum. Dis. 74 (12), 2157–2164. doi:10.1136/annrheumdis-2014-205577.
19. Chung, W. H., Hung, S. I., Yang, J. Y., Su, S. C., Huang, S. P., Wei, C. Y., et al. (2008). Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. Nat. Med. 14 (12), 1343–1350. doi:10.1038/nm.1884.
20. Copaescu A, Gibson A, Li Y, Trubiano JA, Phillips EJ. An Updated Review of the Diagnostic Methods in Delayed Drug Hypersensitivity. Front Pharmacol. 2021;11:573573
21. Demoly P, Adkinson NF, Brockow K, Castells M, Chiriac AM, Greenberger PA, et al. International consensus on drug allergy. Allergy. 2014;**69**:420–437.]
22. Deng Y, Li S, Zhang L, Jin H, Zou X (2018) Association between HLA alleles and lamotrigine-induced cutaneous adverse drug reactions in Asian populations: a meta-analysis. Seizure 60:163–171.
23. Deng Y, Li S, Zhang L, Jin H, Zou X (2018) Association between HLA alleles and lamotrigine-induced cutaneous adverse drug reactions in Asian populations: a meta-analysis. Seizure 60:163– 171.
24. Dibek Misirlioglu E, Guvenir H, Bahceci S, Haktanir Abul M, Can D, Usta Guc BE, Erkocoğlu M, Toyran M, Nacaroglu HT, Civelek E, Buyuktiryaki B, Ginis T, Orhan F, Kocabas CN (2017) Severe cutaneous adverse drug reactions in pediatric patients: a multicenter study. J Allergy Clin Immunol Pract 5(3):757–763. https:// doi. org/ 10. 1016/j. jaip. 2017. 02. 013
25. Dodiuk-Gad RP, Olteanu C, Feinstein A, Hashimoto R, Alhusayen R, Whyte-Croasdaile S, Finkelstein Y, Burnett M, Sade S, Cartotto R, Jeschke M, Shear NH (2016) Major psychological complications and decreased health-related quality of life among survivors of Stevens-Johnsosyndrome and toxic epidermal necrolysis. Br J Dermatol 175(2):422–424.
26. El-Azhary RA, Wang MZ, Wentworth AB, Hickson LJ (2018) Treatment of severe drug reactions by hemodialysis. Int J Dermatol 57(2):177–182.
27. Galvao VR, Aun MV, Kalil J, Castells M, Giavina-Bianchi P (2014) Clinical and laboratory improvement after intravenous immunoglobulin in drug reaction with eosinophilia and systemic symptoms. J Allergy Clin Immunol Pract 2(1):107–110.
28. Gentile I, Talamo M, Borgia G (2010) Is the drug-induced hypersensitivity syndrome (DIHS) due to human herpesvirus 6 infection or to allergy-mediated viral reactivation? Report of a case and literature review. BMC Infect Dis 10:49.
29. Hoetzenecker W, Nageli M, Mehra ET, Jensen AN, Saulite I, Schmid-Grendelmeier P, Guenova E, Cozzio A, French LE (2016) Adverse cutaneous drug eruptions: current understanding. Semin Immunopathol 38(1):75–86.

30. Husain Z, Reddy BY, Schwartz RA. DRESS syndrome: Part II. Management and therapeutics. J Am Acad Dermatol. 2013;**68**:709.e1–709.e9.
31. Iannella G, Greco A, Didona D, Didona B, Granata G, Manno A, Pasquariello B, Magliulo G (2016) Vitiligo: pathogenesis, clinical variants and treatment approaches. Autoimmun Rev 15(4):335–343.
32. Ichihara M, Sobue S, Ito M, Ito M, Hirayama M, Ohno K. Beneficial biological effects and the underlying mechanisms of molecular hydrogen - comprehensive review of 321 original articles. Med Gas Res. 2015;5:12.
33. Jung J-W, Kim D-K, Park H-W, Oh K-H, Joo K-W, Kim Y-S, et al. An effective strategy to prevent allopurinol-induced hypersensitivity by HLA typing. Genet Med. 2015;17(10):807–14.
34. Kinoshita Y, Saeki H. A review of toxic epidermal necrolysis management in Japan. Allergol Int. 2017 Jan;66(1):36-41. doi: 10.1016/j.alit.2016.06.001. Epub 2016 Jul 8. PMID: 27400826.
35. Kocaoglu C, Cilasun C, Solak ES, Kurtipek GS, Arslan S (2013) Successful treatment of antiepileptic drug-induced DRESS syndrome with pulse methylprednisolone. Case reports in pediatrics 2013:928910.
36. Lee HY, Lim YL, Thirumoorthy T, Pang SM (2013) The role of intravenous immunoglobulin in toxic epidermal necrolysis: a retrospective analysis of 64 patients managed in a specialized centre. Br J Dermatol 169(6):1304–1309.
37. Macy E, Ngor E (2014) Recommendations for the management of beta-lactam intolerance. Clin Rev Allergy Immunol 47(1):46– 55.
38. Moon J, Park H-K, Chu K, Sunwoo J-S, Byun J-I, Lim J-A, et al. The HLA-A*2402/Cw*0102 haplotype is associated with lamotrigine induced maculopapular eruption in the Korean population. Epilepsia. 2015;56(10):e161–7.
39. Naisbitt DJ, Nattrass RG, Ogese MO. In vitro diagnosis of delayed-type drug hypersensitivity. Immunol Allergy Clin N Am. 2014;**34**:691–705.
40. Negrini S, Becquemont L (2017) Pharmacogenetics of hypersensitivity drug reactions. Therapie 72(2):231–243.
41. Ng CY, Chen CB, Wu MY, Wu J, Yang CH, Hui RC, Chang YC,Lu CW (2018) Anticancer drugs induced severe adverse cutaneous drug reactions: an updated review on the risks associated with anticancer targeted therapy or immunotherapies. J Immunol Res 2018:5376476.
42. Omenetti S, Pizarro TT. The Treg/Th17 axis: a dynamic balance regulated by the gut microbiome. Front Immunol. 2015.
43. Pavlos R, Mallal S, Ostrov D, Buus S, Metushi I, Peters B, Phillips E. T cell-mediated hypersensitivity reactions to drugs. Annu Rev Med. 2015;66:439-54. doi: 10.1146/annurev-med-050913-022745. Epub 2014 Oct 27. PMID: 25386935; PMCID: PMC4295772.

44. Rothenberger J, Krauss S, Held M, Bender D, Schaller HE, Rahmanian-Schwarz A, Constantinescu MA, Jaminet P (2014) A quantitative analysis of microcirculation in sore-prone pressure areas on conventional and pressure relief hospital mattresses using laser Doppler flowmetry and tissue spectrophotometry. J Tissue Viability 23(4):129–136.
45. Selvan S, Shakir R, Chan A (2013) Pustular vasculitis BMJ Case Reports. https:// doi. org/ 10. 1136/ bcr- 2013- 008806.
46. Shiohara T, Kano Y (2017) Drug reaction with eosinophilia and systemic symptoms (DRESS): incidence, pathogenesis and management. Expert Opin Drug Saf 16(2):139–147.
47. Shiohara T, Mizukawa Y (2019) Drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS): an update in 2019. Allergology international : official journal of the Japanese Society of Allergology 68(3):301–308.
48. Shiohara, T., Mizukawa, Y., and Aoyama, Y. (2015). Monitoring the acute response in severe hypersensitivity reactions to drugs. Curr. Opin. Allergy Clin. Immunol. 15 (4), 294–299. doi:10.1097/ACI.0000000000000180
49. Shirzadi M, Thorstensen K, Helde G, Moen T, Brodtkorb E. Do HLA-A markers predict skin-reactions from aromatic antiepileptic drugs in a Norwegian population? A case control study. Epilepsy Res. 2015;118:5–9.
50. Sousa-Pinto B, Correia C, Gomes L, Gil-Mata S, Araújo L, Correia O, Delgado L. HLA and Delayed Drug-Induced Hypersensitivity. Int Arch Allergy Immunol. 2016;170(3):163-79. doi: 10.1159/000448217. Epub 2016 Aug 26. PMID: 27576480
51. Su, S. C., Mockenhaupt, M., Wolkenstein, P., Dunant, A., Le Gouvello, S., Chen, C. B., et al. (2017). Interleukin-15 is associated with severity and mortality in Stevens-Johnson syndrome/toxic epidermal necrolysis. J. Invest. Dermatol. 137 (5), 1065–1073. doi:10.1016/j.jid.2016.11.034.
52. Syrigou, E., Zande, M., Grapsa, D., and Syrigos, K. (2016). Severe delayed skin reaction during intradermal testing with beta-lactam antibiotics. J. Allergy Clin. Immunol. Pract. 4 (1), 158–159. doi:10.1016/j.jaip.2015.07.018.
53. Tocco-Tussardi I, Huss F, Presman B (2017) Microbiological findings and antibacterial therapy in Stevens-Johnson syndrome/ toxic epidermal necrolysis patients from a Swedish Burn Center. J Cutan Pathol 44(5):420–432.
54. Tomida, E., Kato, Y., Ozawa, H., Hasegawa, H., Ishii, N., Hashimoto, T., et al. (2016). Causative drug detection by drug-induced lymphocyte stimulation test in drug-induced linear IgA bullous dermatosis. Br. J. Dermatol. 175, 1106–1108. doi:10.1111/bjd.14069.
55. Trubiano, J. A., Adkinson, N. F., and Phillips, E. J. (2017a). Penicillin allergy is not necessarily forever. J. Am. Med. Assoc. 318 (1), 82–83. doi:10.1001/jama.2017.6510.
56. Ushigome Y, Mizukawa Y, Kimishima M, Yamazaki Y, Takahashi R, Kano Y, et al. Monocytes are involved in the balance between regulatory T cells and Th17 cells in severe drug eruptions. Clin Exp Allergy. 2018;48(11):1453–63.

57. viard-Leveugle I, Gaide O, Jankovic D et al. TNF-alpha and IFN-gamma are potential inducers of Fas-mediated keratinocyte apoptosis through activation of inducible nitric oxide synthase in toxic epidermal necrolysis. J Invest Dermatol 2013; **133**: 89– 498.
58. voskoboinik, I., Whisstock, J. & Trapani, J. Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol **15**, 388–400 (2015). https://doi.org/10.1038/nri3839
59. Wei CH, Chung-Yee Hui R, Chang CJ, Ho HC, Yang CH, Lin YJ, Chung WH (2011) Identifying prognostic factors for drug rash with eosinophilia and systemic symptoms (DRESS). Eur J Dermatol: EJD 21(6):930–937.
60. Zhang, F. R., Liu, H., Irwanto, A., Fu, X. A., Li, Y., Yu, G. Q., et al. (2013) HLA-B*13:01 and the dapsone hypersensitivity syndrome. N. Engl. J. Med. 369 (17), 1620–1628.
61. Zhang, Jingzhan, et al Jingzhan Zhang, Zixian Lei, Chen Xu, Juan Zhao and Xiaojing Kang. "Current Perspectives on Severe Drug Eruption." Clinical Reviews in Allergy & Immunology, vol. 61, no. 3, Dec. 2021, pp. 282+. Gale Academic OneFile, link.gale.com/apps/doc/A688137068/AONE?u=googlescholar&sid=googleScholar&xid=221cfaf8. Accessed 23 Jan. 2023.