Stamping a Case of Cutaneous Adverse Drug Reaction: Proving Beyond Causality Assessment

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Abstract
Different types of skin testing with a suspected drug have been reported to be helpful in determining the cause of cutaneous adverse drug reactions (CADRs). It is of utmost importance for practicing dermatologists to have a first-hand knowledge of different types of skin tests available in a case of CADR. In each suspected case, a detailed investigation with the suspected agent and correlation of the positive skin reaction with clinical variant of CADR is advocated. Drug skin tests are performed 6 weeks to 6 months after complete healing of the CADR. Drug patch tests are performed similar to the methods used in patch testing in studying contact dermatitis. The commercialized form of the drug used by the patient is tested at 30% dilution. The pure drug is tested at 10% dilution. In severe CADR, drug patch tests should be performed at lower concentrations. It is also of value to test on the most affected site of the initial CADR.

KEY WORDS: Cutaneous adverse drug reaction, intradermal test, patch test, skin prick test

What was known?
CADRs are heterogeneous group of skin reactions and can mimic a plethora of other cutaneous diseases. Skin tests are important tools for the treating physicians to confirm the diagnosis.

Introduction
Cutaneous adverse drug reactions (CADRs) are heterogeneous and can present in varying morphology and severity. The onset of drug reaction can be delayed by months and sometimes occurs even after the withdrawal of the drug. Different drug reactions may mimic a host of noniatrogenic dermatological conditions, and often, we have limited tools for confirming a diagnosis. Even after arriving at a diagnosis, the physicians are challenged to find out the suspected offending agent because of issues of polypharmacy, nonavailability of medical records, over-the-counter use of medicine, and use of indigenous/alternative medicines such as ayurvedic and homeopathic medicines, especially in a country like ours. Definitive guidelines are available internationally for some specific CADR, but often, they are not validated in Indian populations and some are impractical in our socioeconomic context. In such scenario, it is very much needed for a physician to have in-depth understanding of different morphological patterns of CADR and also available laboratory investigations to stamp a case of CADR with reasonable certainty. The present presentation gives an in-depth review of existing tools which can aid clinical judgment in diagnosis of CADR.

Immediate versus Nonimmediate Drug Reaction
It is a common knowledge that drug reactions can be immediate and nonimmediate types. It is important for the clinicians to distinguish between immediate and nonimmediate drug reactions. The former occurs within the 1st h after the last drug administration and is manifested clinically by urticaria, angioedema, rhinitis, bronchospasm, and anaphylactic shock. Nonimmediate reactions occur more than 1 h after last drug administration. The common nonimmediate reactions are maculopapular eruptions and delayed-appearing urticaria/angioedema. In addition, drugs can elicit fixed eruptions, exfoliative dermatitis, acute generalized exanthematous pustulosis (AGEP), drug reaction with eosinophilia and systemic symptoms (DRESS), Stevens–Johnson syndrome, and toxic epidermal

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necrolysis (TEN). Immediate reactions are often the most dangerous; they are IgE mediated and have been extensively studied. On the other hand, the mechanisms involved in the nonimmediate reactions seem to be heterogeneous. In selecting diagnostic tests, it is important to consider whether the reaction is immediate or nonimmediate, as summarized in Table 1.

### Skin Tests

Skin tests are usually the more readily available form of allergy testing for CADR to the physicians. In spite of some limitations, skin tests cannot yet be replaced by in vitro tests. A skin test continues to be essential before beta-lactam exposure. Studies have contributed in recent times in standardizing and proving usefulness of skin testing in the immediate reactions to cephalosporins. Romano et al. demonstrated that skin testing at a concentration of 2 mg/ml in normal saline of several injectable and noninjectable cephalosporins can be a very effective method for evaluating individuals who suffered immediate reactions to cephalosporins. A study involving 128 patients with well-established IgE-mediated allergy to penicillins (mainly to aminopenicillins) supported the advisability of performing skin tests with cephalosporins before their administration to penicillin-allergic patients who require cephalosporin.

Intradermal (ID) and patch tests were performed with the soluble forms of the suspected drugs. Readings were taken at 15–20 min (immediate, prick, and ID), 6–8 h (semi-late and ID), 48–72 h (late, ID and patch test), and an additional hyper-late reading on 6th or 7th day (may be up to 14 days; hyper-late, ID, and patch test), when late readings are negative and the index reaction occurred late. A wheal more than 3 mm (prick) or infiltrated erythema more than 5 mm (ID) is defined as a positive response if there is also a negative response to control solution (0.9% saline) and a positive response to histamine (prick: 1 mg/ml and ID: 0.1 mg/ml). The indications of skin prick test (SPT) and ID test (IDT) are given in Table 2.

### Guidelines in Performing Skin Prick Test and Intradermal Test

A few guidelines have been put forward for performing SPT and IDT with drugs in the investigation of ADR. In a nutshell, the practicing physicians should keep in mind the following points:

1. In general, it is advised to perform the tests 6 weeks to 6 months after the hypersensitivity reaction because it is not known whether or not positive results will persist and whether or not some drug reactivities will last longer.
2. Ideally, immediate skin testing should be performed after an interval that allows resolution of clinical symptoms and clearance of a suspected drug.

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### Table 1: Diagnostic tests for hypersensitivity reactions to drugs

| Type of reaction | Type of test | Name of the test |
|------------------|--------------|------------------|
| Immediate CADR   | In vitro     | Specific IgE assay |
|                  | In vivo      | Flow cytometric basophil activation test |
| Nonimmediate CADR| In vitro     | Lymphocyte transformation or activation test |
|                  | In vivo      | Delayed-reading IDT |
|                  |              | Patch test |

CADR: Cutaneous adverse drug reaction, DPT: Drug provocation test, SPT: Skin prick test, IDT: Intradermal test

### Table 2: Indications for skin prick test and intradermal test in drug allergy workup

| Indications                           |
|---------------------------------------|
| Erythematous eruption/flushing        |
| Urticaria                             |
| Angioedema                            |
| Anaphylaxis                           |
| Conjunctivitis                        |
| Rhinitis                              |
| Bronchospasm/asthma                   |

Late reading of IDT is useful in delayed reactions but bears risk for flare-up reactions to the intradermally applied drug.

### Table 3: ADRs which are not indications for skin prick test and intradermal tests in drug allergy workup

| ADRs                                      |
|-------------------------------------------|
| Drug-induced autoimmune diseases          |
| Bullous pemphigoid                        |
| Pemphigus vulgaris                       |
| Systemic lupus erythematosus              |
| Severe exfoliative skin reactions         |
| Acute generalized exanthematous pustulosis|
| Drug reaction with eosinophilia and systemic symptoms or drug hypersensitivity syndrome |
| Exfoliative dermatitis                    |
| Multilocalized bullous FDE               |
| SJS                                      |
| TEN                                      |
| Severe vasculitis syndromes              |

ADR: Adverse Drug Reaction, SJS: Stevens–Johnson syndrome, FDE: Fixed drug eruption, TEN: Toxic epidermal necrolysis
3. It is important to avoid antihistamines (up to 7 days) before testing.

4. Higher doses of systemic corticosteroids and other immunosuppressive therapy and antidepressants with antihistamine side effects also may interfere and hamper the interpretation of a negative result; hence, these drugs should be stopped for 3 days to 1 month before testing.[11,12,16]

**Drug Provocation Test**

Drug provocation test (DPT) is the controlled administration of a drug to diagnose immune- or nonimmune-mediated drug hypersensitivity and the last step for accurate recognition of drug hypersensitivity reactions when the previous diagnostic evaluations are negative or unavailable. A DPT is performed only if other conventional tests fail to yield conclusive result. In each clinical presentation; “to provoke or not to provoke” a patient should be decided after careful assessment of the risk–benefit ratio. Well-defined benefits of DPT include confirmative exclusion of diagnosis of drug hypersensitivity and provision of safe alternatives. However, there are disadvantages such as safety, difficulty in interpretations of result, lack of objective biomarkers, risk of resensitization, efficiency in daily practice, and lack of standardized protocol, which are needed to be addressed.[7,17]

DPT is generally accepted as the “gold standard” investigation in European perspective for the diagnosis of drug hypersensitivity. However, from the American context, this approach is regarded as graded challenge (or test dosing), defined as the introduction of a medication cautiously so as not to induce a severe reaction. The provocative drug is either an alternative, a structurally/pharmacologically related drug, or the culprit drug itself.[12] A DPT is performed if other less critical or less difficult tests fail to yield conclusive decision. Several factors may influence not only the decision but also the protocol for a DPT, such as the chronology of the index clinical reaction (immediate vs. nonimmediate), the severity of the clinical reaction (anaphylaxis vs. mild reaction), the population involved (child vs. adult), and the facilities of the medical center (including intensive care unit) [Table 4].[7] A definite diagnosis of drug hypersensitivity reaction, in fact, may become a clinical necessity that many drug courses may be required over a lifetime, usually as an emergency. The advantages and the disadvantages of DPT are summarized in Table 5.[18]

DPTs are pivotal to characterize cross-reactivity in drug hypersensitive patients. A search for well-tolerated alternative β-lactam is advised in cases of diagnosed β-lactam allergy.[19] The *in vitro* tests, such as basophil activation test, are not sufficient to differentiate selective reactors and cross-reactors in patients with immediate allergic reactions to β-lactams.[20]

**Table 4: Requirements for a drug provocation test**

| Advantages | Disadvantages |
|------------|---------------|
| Confirmation or exclusion of diagnosis of drug hypersensitivity | Potentially dangerous |
| Less use of more expensive alternatives | DPT protocol is chosen based on patients'/parents' report about the reaction suffered |
| Less use of broad-spectrum antibiotics, decreased risk of antibiotic resistance | False-positive and false-negative results may occur |
| Reduced cost of drug allergy algorithm | Cofactors may be absent |
| Generally, good safety profile | Potential risk of resensitization |
| Acceptable for most patients | Although gold standard, many contraindications to perform DPT may be present |
| Avoidance of unnecessary desensitizations | Lack of standardized protocols, especially for nonimmediate reactions |
| Provision of safe alternative | Subjective symptoms could be difficult to interpret |
| Decreased social burden of drug allergy | Lack of objective and reliable biomarkers (e.g., serum tryptase) |
| | Negative result may not be sufficient to reuse the culprit drug |
| | Need experienced personnel and well-established clinical setting |

**Table 5: Advantages and disadvantages of drug provocation test**

| Advantages | Disadvantages |
|------------|---------------|
| Confirmation or exclusion of diagnosis of drug hypersensitivity | Potentially dangerous |
| Less use of more expensive alternatives | DPT protocol is chosen based on patients'/parents' report about the reaction suffered |
| Less use of broad-spectrum antibiotics, decreased risk of antibiotic resistance | False-positive and false-negative results may occur |
| Reduced cost of drug allergy algorithm | Cofactors may be absent |
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| Decreased social burden of drug allergy | Lack of objective and reliable biomarkers (e.g., serum tryptase) |
| | Negative result may not be sufficient to reuse the culprit drug |
| | Need experienced personnel and well-established clinical setting |

Blanca-Lopez et al. defined a group of patients who were selective responders to amoxicillin but could tolerate penicillin G and penicillin V.[8] In an adult population with a proven β-lactam allergy, 11.9% of patients were sensitized to both penicillins and cephalosporins. The prevalence of cefuroxime allergy was 6.3% (4.2% diagnosed by DPT) in patients sensitive to β-lactams.[21] Zambonino et al. demonstrate,
Table 6: Reported drug patch tests positivity in different reaction patterns

| Reaction pattern                  | Patch test positivity in % |
|----------------------------------|----------------------------|
| AGEP                             | ≥50                        |
| Maculopapular exanthema          | 50-60                      |
| Anticonvulsant hypersensitivity syndrome | 70                      |
| SJS/TEN                          | Up to 100                  |
| Beta lactam antibiotic sensitivity | 39-54                     |
| FDE                              | 40-87                      |
| Contact dermatitis and            | >87                        |
| photocomplex dermatitis           |                            |

AGEP: Acute generalized exanthematous pustulosis, SJS: Stevens–Johnson syndrome, TEN: Toxic epidermal necrolysis, FDE: Fixed drug eruption

using DPT in a group of children with immediate or nonimmediate hypersensitivity to amoxicillin or amoxicillin clavulanic acid, that cefuroxime was tolerated by all patients.[22] Cross-reactivity seems higher in immediate reactions when penicillins and cephalosporins are identical or similar in the R1 side chain.[23]

To sum up, we may conclude that though plugged with various limitations, drug sensitivity tests are important in:

1. Confirmation of diagnosis
2. Exclusion of cross-reactivity of related drugs
3. To provide pharmacologically and/or structurally nonrelated safe alternative
4. Exclusion of drug hypersensitivity
5. The assessment of natural course of drug hypersensitivity reactions.

**Patch Test**

Patch testing with the suspected compound has been reported to be helpful in determining the cause of a CADR and in studying the pathophysiological mechanisms involved. The main advantages of drug patch test are that they can be done with no hospital surveillance because they induce adverse reactions only rarely and that any commercialized form of a drug can be used. It is advised to perform drug patch test after 6 weeks to 6 months following subsidence of the drug rash. Due to the possibility that a low concentration might yield false-negative results, drug patch tests have to be performed with rather high concentrations of the commercialized form of the drug, mostly diluted at 30% in petrolatum and/or in water. However, for some drugs and cases of severe CADR, it is necessary to test with lower concentrations or in other vehicles. Drug patch tests are positive in 32%-50% of patients who have developed a CADR. As false-positive results can be observed, it is always necessary to consider the relevance of any positive drug patch test. Their specificity and their negative predictive value have not yet been determined [Table 6].

**How to Perform Patch Test?**

It is recommended that patch tests are performed initially at diluted concentration of 1%, and if the results are negative, the concentration may be increased up to 10%. Similarly, to avoid false-positive reactions, the content of capsules should be tested at 5% or 10% in petrolatum and not with higher concentrations.

Since the threshold of sensitivity for many pure substances is not yet determined, it is advised to use a 10% concentration in petrolatum and if necessary in other vehicles. If the commercialized form of the drug is tested, pills must have their coating removed. The substance has to be smashed to a very fine powder. This powder can then be tested as it is, but has to be incorporated at 30% in white petrolatum or be diluted at 30% in water. The gel jacket portion of the capsules is moistened and tested as is. Liquid preparations are tested both as is and diluted at 30% in water. With commercialized forms of the drugs, each preparation is done for only one patient and kept not more than 24 h. The name of the chemical form of the molecule (salt, molecule base) has to be noted carefully. Whenever possible, preservatives, coloring agents, and excipients should also be tested, undiluted, or diluted at 10% in petrolatum. In investigating a photosensitivity reaction induced by a drug, both drug patch test and drug photopatch test with the responsible drug have to be performed. The irradiation for drug photopatch test is performed on day 1 with a 5 J/cm² ultraviolet A irradiation.

False-negative results can occur when antibiotics such as beta-lactam antibiotics or pristinamycin[24] are diluted in water, whereas patch tests with the same drug diluted in petrolatum yield positive results.[11]

Testing in the affected area could be of value in certain forms of CADR such as TEN. Klein et al.[25] obtained positive patch tests when co-trimoxazole was tested on the cutaneous sites previously affected by necrolysis while drug patch tests performed on other less affected skin sites remained negative. It could also be of value to test on the highly affected skin sites in maculopapular rashes.

As drug patch test can induce immediate positive reaction, especially with beta-lactam antibiotics, this test has to be read at 20 min, especially in patients who have developed urticaria or anaphylactic shock. Immediate reaction on patch test has been reported with beta-lactam antibiotics, neomycin, gentamycin, bacitracin, and recently with diclofenac.[26]

Because most of the CDRs are related to a delayed cellular hypersensitivity, it is absolutely necessary to do
delayed readings at 48 h, at 96 h, and if negative, on day 7.

The Clinical Significance of Drug Patch Test

When the patch test is negative, it is advisable to perform prick test. If prick test is negative, IDT should be done with immediate readings in urticaria but with both immediate and delayed readings in other CADRs.[11,27] In a study, it was shown that among 60 patients with CADR and negative patch tests with the suspected drug, 35 (58%) had positive results on IDT.[26] Romano et al. found among 94 patients with suspected delayed sensitization to beta-lactam antibiotics, 36% had both positive patch test and IDT, but 8 had positive IDT with negative patch test.[27]

In a CADR due to vancomycin, prick test as well as IDT done with glycopeptide antibiotic remained negative even on delayed reading while drug patch test was positive with specific result.[28] This emphasizes that delayed reactions to IDT may not be sufficient to investigate a CADR.

The usefulness of drug patch test depends on the clinical features of the CADR. In 165 patients suffering from CADR, with a high imputability of one drug, patch test was positive in 33/61 (54%) maculopapular rash, but in only 2/33 (6%) of urticaria and the difference was statistically significant.[29] Patch test was positive in 7/14 AGEP (50%) but only in 2/22 patients with Stevens–Johnson syndrome or Lyell’s syndrome.[30] Patch test is of value in determining the responsible drug in generalized eczema, systemic contact dermatitis, Baboon syndrome, maculopapular rash, AGEP, and fixed drug eruption.[30,31] It also seems to be of value in DRESS. Photopatch test may be useful in studying drug photosensitivity.

On the other hand, it is of less value in investigating urticaria,[24] Stevens–Johnson or Lyell’s syndrome,[30] and pruritus or vasculitis.[30] A large study have reported patch test to be safe and effective in SCAR (Severe cutaneous adverse reactions).

The Usefulness of Drug Patch Test also Depends on the Tested Drug

Drug patch test can be helpful in determining the cause of a CADR. It induces only rarely adverse reactions and can be done with any commercialized form of a drug. A negative patch test does not exclude its role in causing a CADR. Its sensitivity seems to be somewhat lower than the one with IDT; on the other hand, patch test can be positive in patients with negative IDT. Moreover, IDT cannot be done with all the commercialized forms of the drugs. False-positive results can occur and should be considered by testing new products. The specificity and their negative predictive value of patch tests have not been yet determined.

Radioallergosorbent Test

Idiosyncratic drug reactions (IDRs) represent a major health problem, as they are unpredictable, often severe, and can be life-threatening. These are mediated by immunological mechanisms and may contribute up to one-third of all reactions.[32–34] The low incidence of IDRs makes their detection during drug development stages very difficult, causing many postmarketing drug withdrawals and black box warnings. In the recent years, there has been a growing interest in this area of knowledge with an increase in the scientific production and worldwide activities dedicated to it.[35] As IDR is always not predictable based on the drug’s known pharmacology and has no clear dose–effect relationship with the culprit drug, renders diagnosis of IDRs very challenging. The DPT which is an in vivo test considered the gold standard for diagnosis of IDR, but it is not always safe to perform on patients. Radioallergosorbent test (RAST) has the advantage of bearing no potential harm to patients. This review discusses the current role of RAST for diagnosis of IDRs and gives a brief account of their technical and mechanistic aspects. Advantages, disadvantages, and major challenges that prevent these tests from becoming mainstream diagnostic tools are also discussed here.

Adverse reactions to drugs are unintended or undesired effects of a drug therapy that may significantly influence management decisions.[36] The incidence may be as high as 15% in hospitalized patients.[32,33]

Predictable adverse drug reactions are common. These reactions are dose dependent or related to the pharmacology of the drug and include overdose, side effects, secondary or indirect effects, secondary effects related to underlying disease, and drug–drug interactions.[35,36] Unpredictable drug reactions are less common and occur in a small subset of patients. These reactions are not related to the dose or the pharmacology of the drug. Unpredictable reactions include drug intolerance, idiosyncratic reactions, pseudoallergic reactions, and immunologic reactions.[35]

Most assays developed for drug-specific IgE detection including the commercial ones consisted on the quantitation of IgE using radiolabeled anti-IgE antibodies (RAST) or enzyme-linked immunosorbent assay (ELISA) or fluorescent enzyme immunoassay (FEIA) assays. The principle consists of a solid phase to which the hapten conjugated to a carrier protein is bound covalently. The carrier protein can be HSA or other molecules such as polylysines or aliphatic spacers.[37–39] Although HSA has been used for many years,[40] this has not shown to be the most suitable carrier being preferable in many instances, others with a high capacity for hapten fixation and exposition to IgE antibodies.[37–39,41,42] In the recent times, the radiolabeled method has been substituted by the ELISA and the latter with the FEIA although...
no sufficient comparative studies have been made so far. In general, it is accepted that FEIA sensitivity for BPO is reasonable compared to skin testing.[43] This is particularly relevant in the cases of cephalosporins where sensitivity not higher than 20% has been reported.[46] This can be due to the noninclusion of the culprit cephalosporin. For many years, only cefaclor has been available for in vitro testing and it is well known that for this beta-lactam the side chain at R1 position is very important in the specific IgE recognition.[45-47] Similar assays have been developed for other drugs, being in most cases experimental prototypes that need further validations in a sufficient number of positive controls.[48-55]

An alternative solid phase used for many drugs has been epoxy-activated sepharose to which drugs bind covalently. Classical drugs used have been cephalosporins, quinolones, and muscle relaxants with different sensitivity and specificity results.

A RAST is a blood test using radioimmunoassay test to detect specific IgE antibodies, to determine the substances an individual is allergic to. Hence, RAST can be a very specific and important. Although the radioactive technique is no longer used, “RAST” has become a generic name for the technique. The term RAST was originally a brand name, but it is now often used colloquially (though incorrectly) to describe any in vitro assay for allergen-specific IgE.

The Phadebas RAST (Pharmacia, Uppsala, Sweden) was the first assay reported for the detection of the allergen-specific IgE antibody.

The RAST is a solid-phase radioimmunoassay that was first developed in 1967. It measures circulating allergen-specific IgE antibodies. RAST and RAST analogs are performed by linking the allergen (i.e., drug) in question to a solid phase (i.e., carbohydrate particle, paper disk, or the wall of polystyrene test tubes or plastic microtiter wells).[56,57] The drug attached to the solid phase is incubated with the patient’s serum, during which time specific antibodies of all immunoglobulin isotypes are bound. After washing, a second incubation is done with a radiolabeled, highly specific anti-IgE antibody. Another washing is performed, and the bound radioactivity is then directly related to the drug-specific IgE antibody content in the patient’s serum. The results from the serum under the study are compared with a positive reference serum and a negative control serum. The results are reported in arbitrary units of IgE per milliliter of serum.

Application of RAST for diagnosing a drug allergy is unfortunately limited because of incomplete knowledge of the conformation of most drugs and their metabolites.[54] Solid-phase immunoassays have been developed to detect serum IgE antibodies directed against the penicilloyl epitope (i.e., the major penicillin determinant). At present, there is no in vitro RAST for the minor penicillin determinant antibodies.[54] Direct detection of a drug-specific IgE in patient blood is a strong indicator of an immune reaction, but this is not necessarily true in all cases. An individual may have circulating IgE that recognizes a drug molecule without having an immune reaction toward that drug.

**Lymphocyte Proliferation Tests and Cytokine Measurements**

In vitro assessment of this lymphocyte activation may help in early identification and in vitro assessment of interferon-gamma can help as an adjunct tool.

**Conclusion**

It is important for the treating physicians to have a thorough knowledge of skin tests used in the CADR, and when used correctly and judiciously, these tests can help the physicians to confirm various clinical types of CADR.

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**Conflicts of interest**

There are no conflicts of interest.

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### What is new?

A thorough knowledge of the latest concepts in skin testing allows the practitioner to confirm and validate cases of CADRs.

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