Skin Tests in the Diagnosis of Drug Hypersensitivity Reactions

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Abstract: Adverse drug reactions (ADRs) are an area of concern for pharmaceutical drug development. Among these, drug hypersensitivity reactions are neither dose-dependent nor predictable, and affect only predisposed individuals. Clinical and immunological studies suggest that IgE-mediated (type I) and cell-mediated (type-IV) pathogenic mechanisms are involved in many immediate (i.e., occurring within 1 hour after the last drug administration) and non-immediate (i.e., occurring more than 1 hour after the last drug administration) hypersensitivity reactions, respectively. Skin prick, patch, and intradermal tests are the most readily available tools for the evaluation of hypersensitivity drug reactions. The diagnostic value of skin tests for many drugs still has not been fully established. Reliable skin test procedures for the diagnosis of drug hypersensitivity have been defined, and test concentrations have been validated for many drugs. Skin tests should be carried out according to the clinical features of ADRs. In immediate drug reactions, an IgE-mediated mechanism can be demonstrated by a positive skin prick and/or intradermal test after 20 minutes, whereas in non-immediate reactions, a T-cell involvement can be found by a positive patch test and/or a late-reading intradermal test. The predictive value of skin tests varies with the drug tested and is especially high with β-lactams, muscle relaxants, insulins, platinum salts, streptokinase, and chymopapain. Further diagnostic tests are required in the assessment of drug hypersensitivity reactions. However, skin tests can provide information about the culprit drug and the mechanism involved in certain reactions. The present review addresses literature data regarding the diagnosis of drug hypersensitivity reactions by skin tests.

Key Words: Drugs, hypersensitivity, skin tests, patch tests, prick tests, intradermal tests, allergy diagnosis.

INTRODUCTION

The available diagnostic procedures for evaluating an adverse drug reaction (ADR) are the history, skin tests, *in vitro* tests, and provocation tests [1]. In the field of drug hypersensitivity reactions, it is particularly difficult to come to a reliable diagnosis. The history is not always reliable, different drugs are often taken simultaneously, and the clinical picture of drug hypersensitivity reactions is very heterogeneous. Furthermore, drug metabolites may be responsible for some adverse reactions, many test reagents are standardized for neither *in vitro* nor *in vivo* (skin) tests, and provocation tests are cumbersome and dangerous. Thus, formerly most doctors relied only on the history and a few reference manuals for ADR diagnosis, without attempting to investigate the relationship between drug intake and symptoms or to clarify the mechanism of the reaction. Such an attitude led to a lack of research in this highly relevant field [2].

However, skin tests are powerful tools for the evaluation of a hypersensitivity drug reaction, but their diagnostic value for many drugs still has not been fully established. Moreover, skin tests give insights concerning the immunologic pathogenic mechanism. In recent years, members of the ENDA (European Network on Drug Allergy), the European Academy of Allergology and Clinical Immunology interest group on drug hypersensitivity, has developed useful simple test procedures for the diagnosis of hypersensitivity reactions to many drugs [2-6]. General principles for skin testing with drugs and establishing the best concentrations for already well-studied substances have been formulated. This article summarizes general principles of skin testing with drugs and provides diagnostic protocols for the most important drugs responsible for hypersensitivity reactions.

CLASSIFICATION OF ADVERSE DRUG REACTIONS AND SELECTION OF PATIENTS FOR SKIN TESTING

Although patients who have experienced ADRs often refer to them as "drug allergies", in fact, few ADRs are truly allergic [1]. About 80% of all ADRs are common, predictable reactions that can occur in any given individual and are related to the pharmacological activity of the drug [7]. These reactions, also termed "type A reactions" [8], are dose-related and reversible by dose reduction or withdrawal. Drug hypersensitivity reactions, also termed "type B reactions" [8], are less common, but can be severe and even fatal. They are neither dose-dependent nor predictable, and affect only predisposed individuals. Included in this category are drug intolerance (pharmacological toxicity of a drug at therapeutic dosages), idiosyncratic reactions (non-immunological hypersensitivity that can not be explained by the pharmacological properties of a drug), and drug allergy (hypersensitivity with the involvement of one or more immunological mechanisms). When idiosyncratic reactions cause symptoms similar to allergic ones, the term "pseudo-allergy" may be used

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[1, 9]. According to the Nomenclature Review Committee of the World Allergy Organization [10], however, hypersensitivity reactions for which a definite immunological mechanism, either IgE- or T-cell-mediated, is not demonstrated should be called non-allergic hypersensitivity reactions.

Allergy tests, such as skin tests and *in vitro* tests, generally give positive responses only in allergic ADRs, where they are able to demonstrate an immunological mechanism. Unfortunately, allergic drug hypersensitivity reactions often can not be clinically differentiated from non-allergic ones. Factors characterizing the former reactions may be the presence of a prior sensitization period, reactivity to low dosages of the drug, and typical manifestations, such as urticaria and anaphylaxis immediately after administration of a drug [2]. A list of common clinical manifestations for which skin testing could be useful is shown in Table **1**. In ADRs, however, this general scheme is often unreliable. Sensitization may be unapparent and non-allergic hypersensitivity reactions may mimic symptoms of allergy. ENDA members currently test patients with a number of drugs to gain and publish experience on the value of skin testing under different conditions. In many patients, no allergy will be proven, which may be due to the lack of either adequate test reagents or procedures, or may indicate a non-allergic mechanism and should encourage further research. There are some manifestations of ADRs where immunological mechanisms could be involved, but skin testing has generally not been considered helpful. For example, the value of skin tests in hematological (e.g., anemia, thrombocytopenia, leukopenia), renal (e.g., glomerulonephritis), hepatic (e.g., hepatitis) manifestations, or autoimmune diseases, such as systemic lupus erythematous, bullous pemphigoid, pemphigus vulgaris, and interstitial lung disease, has not been proven [2].

Table 1. Typical Clinical Manifestations in which Skin Testing could be Useful for the Diagnosis of Drug Hypersensitivity Reactions (Adapted from Brockow *et al.* [2])

Immediate reactions
Anaphylaxis
Bronchospasm
Conjunctivitis
Rhinitis
Urticaria/angioedema
Non-immediate reactions
Acute generalized exanthematous pustulosis
Contact dermatitis
Erythema multiforme
Exanthematous drug eruptions
Fixed drug eruptions
Photoallergic reactions
Purpura/leukocytoclastic vasculitis
Stevens-Johnson syndrome
Toxic epidermal necrolysis

On the other hand, performing skin prick tests, (SPTs), intradermal tests (IDTs), and/or patch tests (PTs) often produces positive results and is especially recommended in hypersensitivity reactions to β-lactam antibiotics (mainly penicillins and cephalosporins), muscle relaxants, insulins, streptokinase, and chymopapain [2], as well as in delayed local or exanthematous reactions to non-β-lactam antibiotics, carbamazepine, pyrazolones, and tetrazepam [2, 4, 11-16].

GENERAL PRINCIPLES

In all immediate ADRs, such as urticaria/angioedema, rhinitis, conjunctivitis, bronchospasm, and anaphylaxis, SPTs and IDTs are generally recommended [2]. An algorithm for the use of skin tests is given in Fig. (**1**). However, it has to be considered that skin tests may be regarded as minimally invasive provocation tests and systemic reactions during skin testing might occur. Patients who have been hospitalized because of allergic reactions and/or life-threatening hypersensitivity reactions, including anaphylaxis, are at risk, even if there is a long time interval between the drug hypersensitivity reaction and skin testing [17]. A risk/benefit analysis has to be made of the allergologic workup, specifically regarding the importance of the drug, the risk for the patient, and any treatments for possible adverse reactions. Pregnant women should not be tested. In patients at risk, the drug should initially be tested with a high dilution of the test preparations (e.g., 1/1,000-1/100,000) [2]. The next concentration should be applied only after the highest dilution has yielded a negative result. Although not validated, an open application can also be performed initially in severe immediate reactions. Emergency treatment should be available, since systemic side effects may occur, specifically in β-lactam hypersensitivity diagnosis, in up to 11% of cases [18].

IDTs are useful diagnostic tools in non-immediate ADRs, such as contact dermatitis, photo-contact dermatitis, exanthematous eruptions, urticaria/angioedema, purpura pigmentosa progressiva, leukocytoclastic vasculitis, fixed eruptions, erythema multiforme, acute generalized exanthematous pustolosis (AGEP), drug reaction (or rash) with eosinophilia and systemic symptoms (DRESS), also called hypersensitivity syndrome, Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN), PTs and/or late-readings (i.e., after 24, 48 and 72 hours) [4, 11-16]. Systemic reactions following skin testing have also been reported in the evaluation of non-immediate hypersensitivity drug reactions [19]. In severe, non-immediate reactions it is advisable to extend the time interval between tests and not to perform IDTs with high concentrations before performing PTs. Even PTs should be done with concentrations lower than those routinely used [15]. Especially in very severe skin reactions (e.g., TEN, SJS, DRESS, severe bullous exanthemas, and vasculitis), skin testing should be performed with caution and after risk/benefit analysis. However, a recurrence or elicitation of a TEN due to skin testing has not been described in the literature [20].

METHODS

SPTs, IDTs, PTs, and photopatch tests with many drugs are well standardized skin testing methods. Scratch tests and open PTs are used in some centers, but are neither adequately standardized nor validated.

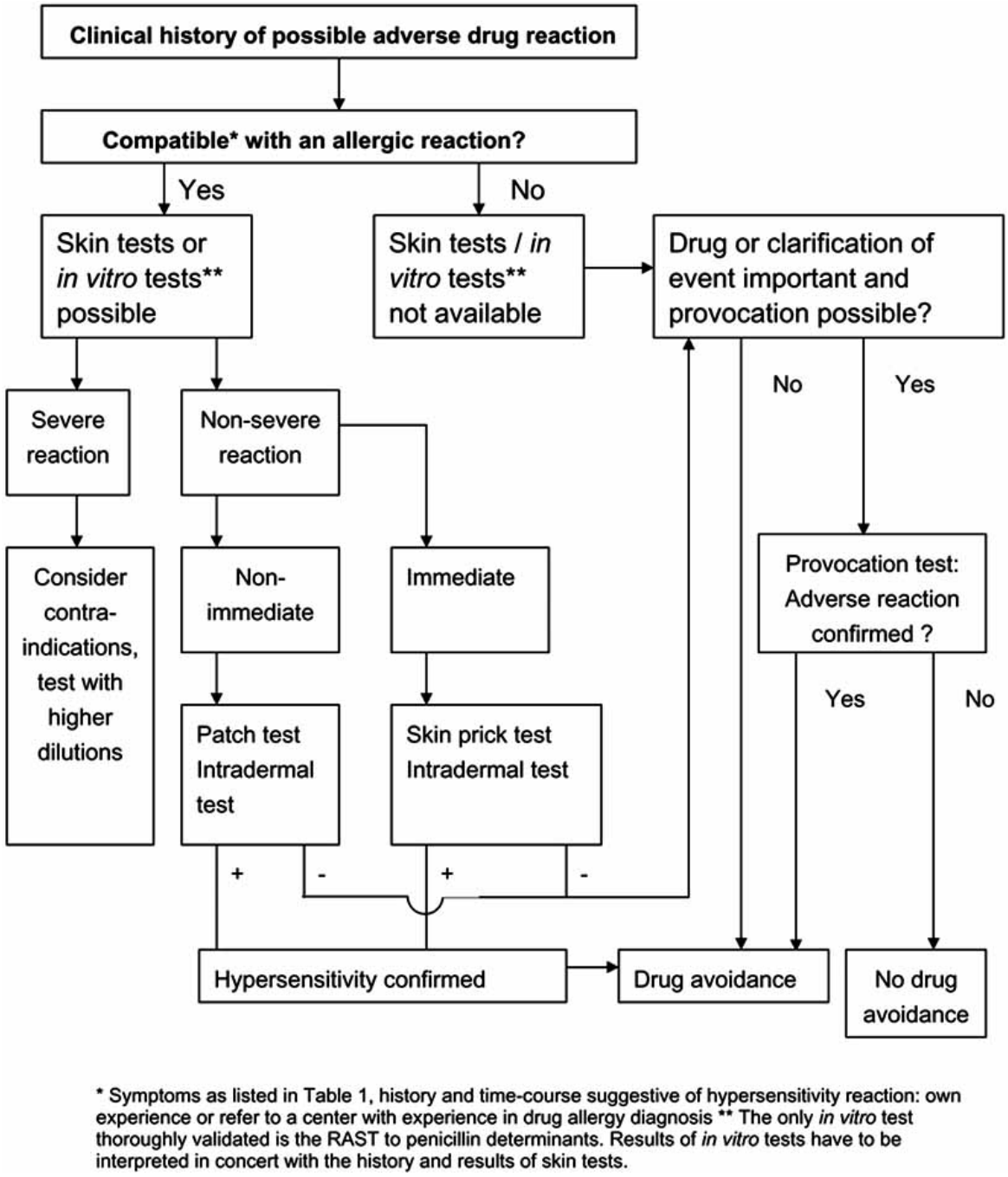

Fig. (1). Algorithm for the use of skin tests in the diagnosis of drug hypersensitivities (adapted from Brockow *et al.* [2]).

In selecting diagnostic tests, it is important to consider whether the reaction is immediate or non-immediate [21]. SPTs and immediate-reading IDTs are indicated for the former reactions, while PTs and delayed-reading IDTs are useful in evaluating non-immediate ones.

Skin Test Procedures

a) Skin Prick Tests and Intradermal Tests

A SPT is done by pricking the skin with a special device through an allergen solution so that the allergen penetrates into the epidermodermal junction zone [2]. It is the safest and easiest skin test, but is only poorly to moderately sensitive for diagnosing drug hypersensitivity reactions. An IDT is performed by injecting 0.02-0.05 ml of an allergen intradermally, which raises a small bleb. The IDT is more sensitive than the SPT; however, it also entails a higher risk of inducing an irritative, falsely positive reaction when injected at too high concentrations.

Certain drugs, which could decrease the skin test response (Table **2**), must be discontinued prior to skin testing. At the time of testing, patients should be free of infectious or inflammatory diseases, unless skin testing is urgently needed. If the drug to be tested has induced an anaphylactic reaction, the intake of β-blocking agents should be discon-

tinued according to their half-life of elimination (usually for 48 hours), as these drugs may interfere with the treatment of a possible systemic reaction elicited by the skin test.

Table 2. Drug-Free Intervals Recommended for Drugs which can Decrease Skin Test Reactivity

Immediate reactions	
H1-antihistamines	5 days
Imipramines, phenothiazines	5 days
β-adrenergic drugs	none
Glucocorticoids	
- long-term, ≤10 mg prednisolone equivalent	none
- long-term, >10 mg prednisolone equivalent	3 weeks
- short-term, ≤50 mg prednisolone equivalent	3 days
- short-term, >50 mg prednisolone equivalent	7 days
Topical corticosteroids in test area	7 days
Non-immediate reactions	
Glucocorticoids	
- long-term, >10 mg prednisolone equivalent	3 weeks
- short-term, ≥50 mg prednisolone equivalent	1 week
- short-term, ≤50 mg prednisolone equivalent	none
Topical corticosteroids in test area	2 weeks

SPTs are performed on volar forearm skin. Normally these tests are well tolerated, but in highly IgE-sensitized patients generalized symptoms (from urticaria to anaphylaxis) might occur [22]. If the SPT is negative after 15-20 minutes, an IDT can be performed, generally on the volar forearm, although other regions, such as the upper back, can be tested [2, 15]. Unfortunately, there is no comparison for drug allergens between these regions. When injected intradermally at a volume of 0.05 ml and a concentration of 1 mg/ml and 0.1 mg/ml, respectively, the positive controls histamine and codeine produced a larger wheal on the back than on the forearm [23]. The painfulness of IDTs limits their use in young children.

b) Patch Tests

In a PT the allergen is fixed on the back of the patient by a tape for a period of 1-2 days and the result is read after one and two to three days [2, 15]. A photopatch test is a modification of the PT used when photoallergic or phototoxic reactions are suspected [24]. The PT is removed and the skin is irradiated with ultraviolet (UV) light, 5 or 10 J/cm^2 UVA, after one day. The readings are performed after 2, 3, and 4 days.

Immediate reactions to the drug should be ruled out by the clinical history before the PTs are applied.

Because the test reactivity is usually diminished after prior strong UV exposure, patch testing should be done at least four weeks after extensive UV exposure, e.g., after holidays at the seaside [25]. Drug-free intervals for certain drugs that could influence the test results are listed in Table **2**. The patient should also be free of infectious diseases, fever, or inflammatory reactions at the time of testing.

PTs are done on unaffected, untreated and uncleaned skin of the upper back using aluminium cups (“Finn Chambers”) or an equivalent fixed with a "hypoallergic" tape.

Skin Test Documentation and Scoring

a) Skin Prick Tests and Intradermal Tests

SPT and IDT readings should be done after 15-20 min, if immediate reactions are analyzed. These reactions are documented by measuring the mean diameter of the wheal and erythema at the site of the test substances, as well as of the positive (histamine) and negative (normal saline) control, immediately and 15-20 minutes after the injection. Many qualitative scoring systems are available and are used in different centers. In order to compare the results, a morphological score should be applied as well, enabling different scoring systems to be compared. The best documentation method is to outline the size of the injected area and of the reaction at 15-20 minutes on a translucent cellophane tape. Criteria for positivity differ among centers [2, 6, 13]. According to the criterion defined by the ENDA [2, 3], reactions are considered positive when the size of the initial wheal increases by 3 mm or more in diameter after 15-20 minutes and is associated with a flare. For clinical studies, the mean diameter is recorded by measuring the largest and the smallest diameter at right angles to each other. Both diameters are recorded, summed and divided by 2. For research studies, the area can also be determined by other, more precise methods (cellophane weighing, planimetry, and computerized scanning) [26].

For the evaluation of non-immediate (late) reactions, skin tests are also read after 24 and 72 hours [2, 4, 27, 28]. As time intervals between testing and positive test reactions may vary, additional readings may be necessary and the patient should be instructed to return to the center, if strong itching occurs, or reactions become visible as soon as after 6 hours or as late as after 96 hours or more [2]. Late reactions should always be examined by a physician. They are documented by the diameter of the erythema and the papulation/infiltrate, as well as by a morphological description (erythematous swelling, erythematous infiltrate, only erythema, eczema with papulation ± vesicles). Any infiltrated palpable erythema is considered to represent a positive reaction. For comparison and research purposes, photo-documentation and, if possible, histology are recommended, as there is currently no widely accepted standard for the detailed positivity criteria (e.g., diameter of erythema, degree of papulation).

b) Patch Tests

PT readings should be done at least two times: 15 minutes after removal of the strips and 24 hours later [2]. Additional readings after 96 and more hours might also be needed in some cases. Reactions may also occur earlier or much later (as in the case of corticosteroids and phenylephrine). Patients should be instructed to inform the doctor of any later reaction. Scoring is done according to the PT and photopatch

test classifications of the European Environmental and Contact Dermatitis Research Group (Table **3**).

TEST PREPARATIONS, TEST VEHICLES, AND SOLUTIONS

For most drugs, commercial skin test preparations are not available. Specific standardized skin test reagents are available only for β-lactams. In effect, kits containing penicilloyl-polylysine (PPL) and minor determinant mixture (MDM) were developed by Allergopharma (Allergopen™, Steinbeck, Germany) and Hollister-Stier (PrePen™, Spokane, WA, USA) for skin testing patients with suspected hypersensitivity reactions to these antibiotics, and validated diagnostic procedures have existed for many years [3, 4, 29]. Because of marketing reasons, however, these diagnostic kits were withdrawn from the markets in Europe as well as in the USA. Nevertheless, a new kit (DAP™, Diater, Madrid, Spain) is commercially available and appears to be comparable to the Allergopharma one; however, it is licensed only in a few European countries. For all other drugs, the test material has to be drawn from the drug preparations available on the market. In order to achieve adequate standardization, skin testing should be performed with injectable compounds, such as those used for the parenteral route. They should normally be diluted in 0.9% NaCl. For non-hydrosoluble drugs, a stock solution can be prepared in dimethyl-sulphoxide (DMSO) and further diluted with 0.9% NaCl [2]. A negative control with the same concentration of DMSO is needed in these cases.

Drugs which are not available in a soluble form (e.g., only tablets) may be tested by SPTs and/or PTs after the tablets have been smashed in a mortar and diluted with 0.9% NaCl or petrolatum [12, 13]. In this case, the tablets should be weighed and the concentration of the active ingredient determined. Test concentrations should be noted in mg of drug/ml of vehicle. Optimal test concentrations for most drugs are still unknown.

For IDTs, sterile solutions are obligatory. For non-injectable drugs, the powder contained in capsules or obtained by removing the external layer of tablets with a scalpel can be used in intradermal testing. After weighing the powder, solutions can be prepared under a laminar flow, and they can be sterilized by filtration through single-use devices (Minisart™ NML Syringe Filter, 0.20 μm, Sartorius AG, Goettingen, Germany), as previously described [30].

For PTs, substances may be diluted in 0.9% NaCl or, depending on the solubility and toxicity of the preparation (e.g., with acidic preparations), in petrolatum. Table **4** shows concentrations commonly used in patch testing with different drugs.

Patch tests can give false negative results, mainly because of a poor penetration of the drug into the epidermis. For this reason, it is crucial to use different vehicles like petrolatum, water, and alcohol. However, there are few data in the literature comparing such vehicles in patch testing with specific drugs such as penicillins [31].

Finally, in case of positive reactions to PTs with drugs in the form of syrups, pills, and capsules, preservatives, coloring agents and excipients should also be tested.

DETERMINATION OF TEST CONCENTRATIONS

Only a skin test reaction to a drug tested at a concentration that does not cause an unspecific irritative reaction in a sufficient number of controls is indicative of drug hypersensitivity. Concentrations used empirically for testing specific drugs can be found in the literature [2, 13, 15]. If optimal skin test concentrations for a given drug are not known, one may initially test the compound at a concentration similar to that of a related drug for which there are literature data. Ideally, molar concentrations of the drug should be given, but in practice concentrations in mg of drug/ml of vehicle are normally used.

IDTs with many undiluted drugs produce irritative responses in both patients and controls, whereas this phenomenon is seldom observed in prick testing. Thus, the optimal test concentration has to be known or has to be determined for each drug. The optimal test concentration is the highest concentration of a particular drug that does not induce any irritative skin reactions in subjects who have never been exposed, as well as in subjects who have been exposed and have tolerated them, but may produce positive results in patients with drug hypersensitivity. A specificity higher than 95% should be attempted. Test solutions, e.g., for penicillins,

Table 3. Scoring of Patch Test Reactions (Adapted from Brockow *et al.* [2])

Clinical Picture	Score	Conclusion
Faint erythema only	? or +?	Doubtful reaction
Erythema, infiltration, possibly discrete papules	+	Weak positive reaction
Erythema, infiltration, papules, vesicles	++	Strong positive reaction
Intense erythema, infiltration, coalescing vesicles	+++	Extreme positive reaction
	-	Negative reaction
	IR	Different irritative reactions
	NT	Not tested

« +, ++, +++ » are regarded as positive skin test reactions and « –» as a negative skin test reaction.

Table 4. Drug Concentrations Commonly Used in Patch Testing

Antibiotic	DKG[1]	De Groot [33]	Barbaud [13]	Others [12, 27]
Penicillin G	5% pet	Pure 1% pet 10000 iU pet	Pure in powder with sodium citrate*	Romano: 5000 iU/g pet Bruynzeel: 20% w/w
Other penicillins	5% pet	Pure 1% pet	Pure in powder*	Romano: 5% pet (20 controls) Bruynzeel: 20% w/w
Cephalosporins	5% pet	20% pet or pure 0.5% water	Pure in powder*	Bruynzeel: 20% w/w
Cotrimoxazole	Trimethoprim 5% pet Sulfamethoxazol 5% pet	Sulfonamide (not specified): 5% pet	80 mg/ml in water	
Tetracycline- HCl	2% pet	3% pet 5% pet	Doxycycline: 20 mg/ml in water	
Gentamycin sulfate	20% pet	20% pet		
Ciprofloxacin, ofloxacin	5% pet		Norfloxacin: in powder from pill*	
Erythromycin	1% pet	1% pet 5% pet 10% pet	Pure in powder*	
Pristinamycine			Pure in powder*	

[1] DKG: German contact allergy group (test concentrations in the German practice).
* All these preparations were tested pure and diluted to 30% in water and in petrolatum.
pet= in petrolatum (vaselin), w/w=water solution.

have to be prepared every day from the intravenous form under sterile conditions [3]. Most muscle relaxants may be stored for 3 months [6]. The shelf life of test solutions has not been investigated for most drugs.

In immediate reactions, gradually increasing concentrations are tested first in patients and afterwards in controls to titrate the concentration causing a positive test reaction [2]. Appropriate concentrations for testing with fresh preparations have not been well studied in large groups of patients and controls. However, a study by Empedrad *et al.* [32] found non-irritating intradermal skin test concentrations for several antibiotics, including β-lactams, quinolones, and macrolides.

Initially the SPT is done at a low concentration (usually 1/100 of the intravenous preparation) and, if no reaction occurs, the concentration is increased tenfold each time until a positive reaction occurs. If no reaction is elicited in the SPT, the IDT starts normally with a dilution of 1/100 of the SPT concentration and the concentration is increased until the final one is reached. The latter should be validated in controls. However, this practice is increasingly difficult for ethical reasons, as an ethical committee approval is often required for all these tests in controls.

In non-immediate reactions, some PT concentrations have been published (Table **4**) [12, 13, 27, 33]. Such concentrations are often of 1-5% [15]. False positive results have been observed by Barbaud's group in patch testing with colchicine at 10% in petrolatum, misoprostol at 30% in petrolatum, and drugs containing sodium lauryl sulphate [15]. Therefore, there is a need for further validation of the optimal vehicle and test concentrations.

TIME POINT OF SKIN TESTING

Generally, skin tests should be performed after a time interval which allows resolution of clinical symptoms, as well as clearance of the suspected drugs and of anti-allergic medications. It is known that in case of immediate reactions the sensitivity of skin tests with some drugs, such as penicillins, decreases with time [3]. Thus, in case of negative results of allergologic tests, including challenges, a second evaluation is advisable [3]. There are few data on the optimal time interval between the clinical reaction and skin testing. Generally, a time interval between 1 and 6 months has been suggested. A recent study concerning hypersensitivity reactions to iodinated contrast media (ICM) [34], analysing cross-sectional data, concluded that the highest frequency of positive responses to skin tests was between 2 and 6 months after the reaction.

INTERPRETATION OF SKIN TEST RESULTS

As for positive skin tests with other allergens, the result should be always interpreted together with the clinical history and with *in vitro* test results, when available [35]. The negative predictive value of skin tests is low for most drugs. For some drugs, metabolites rather than the active drug itself may be responsible for the reaction [36]; others drugs act as haptens, which have to be conjugated with a carrier protein

before becoming an allergen [37]. A negative skin test to a drug alone is unreliable in ruling out drug hypersensitivity. Provocation tests should be considered in case of a negative skin test, after evaluating the risks and the benefits for the patient (Fig. **1**) [38].

On the other hand, when a proper technique and proper drug concentration have been employed, a positive skin test result allows an allergic hypersensitivity to be diagnosed. The positive predictive value of a skin test tends to be high. By means of skin tests, many hazardous provocation tests can be avoided. In case of a specific reaction to a skin test, together with a corresponding clinical history, the patient may be advised to avoid the drug concerned and those potentially cross-reactive (Fig. **1**), and an allergy pass should be issued.

Standardized Skin Test Procedures for Specific Drugs

β-Lactam Drugs

About 10% of patients report a penicillin allergy; however, only 10% to 20% of them are truly allergic when assessed by skin testing [39]. Many studies have provided evidence of the usefulness of skin tests to diagnose β-lactam allergy, as well as information on validated test concentrations and on the sensitivity and specificity of skin testing [13, 18, 27, 28, 40].

Other studies have emphasized that the clinical history in patients reporting adverse reactions to β-lactams is not reliable and thus not predictive of subsequent skin test results [41-43]. In both the ENDA position papers [3, 4] and the American practice parameters [29], skin testing with PPL and MDM represents the first method for diagnosing both immediate and non-immediate hypersensitivity reactions to β-lactams. Moreover, recent studies have emphasized the importance of skin testing with PPL and MDM in diagnosing β-lactam hypersensitivity [44, 45]. Bousquet *et al.* [44] observed positive responses to skin tests in 136 (16.5%) of 824 patients with histories of β-lactam hypersensitivity; 20 (14.7%) of them were positive only to PPL and/or MDM. Matheu *et al.* [45] diagnosed an IgE-mediated hypersensitivity in 44 (9.5%) of 463 patients with such histories; 21 (47.7%) of the sensitive patients displayed positive skin tests only to PPL and/or MDM. As previously mentioned, unfortunately, Allergopharma and Hollister-Stier stopped the production of PPL and MDM in 2004, and this is hampering the diagnosis of β-lactam hypersensitivity [44-46]. Nevertheless, penicillin reagents (PPL and MDM) have been sold in Spain by Diater (DAP™) since 2003 as an allergen for prick and intradermal tests. In two studies [47, 48], a good concordance between the Allergopharma reagents (Allergopen™) and the DAP™ ones was observed. Specifically, in the study by Romano *et al.* [48], Allergopen™ MDM and DAP™ MDM produced identical results in all 195 patients evaluated, 22 of whom were positive to both reagents. Results of skin testing with PPL were concordant in 190 (97.4%) of the 195 subjects. The DAP™ kit, however, has not yet been officially approved by the authorities of several European countries.

Not only the central core structure of the β-lactam, but also side chain structures are able to elicit allergic responses [49]. Thus, in skin testing subjects with hypersensitivity reactions to β-lactams, the use of benzyl-penicillin, amoxicillin, ampicillin, and any other suspected β-lactam, in addition to PPL and MDM, is recommended [3, 4, 50]. Skin test concentrations are given in Table **5**. In case of negative responses to allergologic tests, including *in vitro* ones, a provocation test with the suspected drug can be performed [3, 4, 38].

Table 5. Skin test Concentrations Recommended for Both Prick and Intradermal Tests with β-Lactams

Hapten	Dose	Units
BPO*	$5 \cdot 10^{-5}$	mmol/L
MDM**	$2 \cdot 10^{-2}$	mmol/L
Amoxicillin	20–25	mg/ml
Ampicillin	20–25	mg/ml
Any other penicillin	20–25	mg/ml
Any cephalosporin	2	mg/ml
Imipenem/cilastatin	0.5/0.5	mg/ml
Meropenem	1	mg/ml
Aztreonam	2	mg/ml

*initial dilution is 1/10 (or even more diluted in severe reactions)
**initial dilution is 1/100 (or even more diluted in severe reactions)

The specificity of skin tests with all β-lactam drugs is very good, reaching 97% to 100% [3, 28]. With regard to their sensitivity, in one study encompassing 290 patients with histories of immediate urticarial and/or anaphylactic reactions to penicillins [18], the sensitivity of skin testing was 22% for PPL, 21% for MDM, 43% for amoxicillin, and 33% for ampicillin. Most subjects were skin test positive to more than one penicillin reagent, and the combination of all four haptens gave a sensitivity of 70%. In another study [28], which evaluated 241 subjects with non-immediate reactions to penicillins, patch tests with benzylpenicillin were positive in 7.5% of patients, while ampicillin and amoxicillin elicited positive reactions in 37.3%. Delayed-reading intradermal tests with MDM and benzylpenicillin were positive in 12% of cases, while those with ampicillin and amoxicillin were positive in 39%. However, considering only the 166 subjects with aminopenicillin-associated maculopapular exanthema, the sensitivity of patch tests and delayed-reading intradermal tests with both ampicillin and amoxicillin was 52.4% and 54.2%, respectively.

As far as β-lactams other than penicillins are concerned, skin testing with parent cephalosporins has proved to be a reliable method for diagnosing IgE-mediated reactions to them [30, 51-53]. In a study that evaluated 76 adults with immediate reactions to cephalosporins [30], the rate of positive responses to skin tests with the responsible drugs at a concentration of 2 mg/ml 0.9% NaCl was 69.7%; it increased to 78.9% when considering also the results of the re-evaluation of subjects with negative results in the first aller-

gologic workup who underwent challenges. In another study concerning immediate reactions to cephalosporins [52], however, the sensitivity of cephalosporin skin testing was 30.7% (39 of 127 patients). Therefore, further studies should be performed in large samples of subjects with immediate reactions to cephalosporins in order to fully establish cephalosporin skin test sensitivity.

Cephalosporin skin tests also help to find safe alternatives. In a study regarding 128 patients with a well-established IgE-mediated allergy to penicillins [54], all 101 patients who displayed negative skin tests for cefuroxime, ceftazidime, ceftriaxone, and cefotaxime and underwent graded challenges with cefuroxime axetil and ceftriaxone tolerated them. Skin testing with native carbapenems is also useful in finding safe alternatives in penicillin-allergic subjects. In effect, three studies [55-57] found a 0.9% rate of positive responses to skin tests with imipenem/cilastatin (at a concentration of 0.5 mg/ml 0.9% NaCl for each component) and meropenem (at a concentration of 1 mg/ml 0.9% NaCl) among samples of adults or children larger than 100 subjects with a well-demonstrated IgE-mediated hypersensitivity to penicillins. In these studies [55-57], all negative subjects who agreed to undergo imipenem/cilastatin and/or meropenem challenges tolerated them; specifically, 42 adults tolerated imipenem/cilastatin, 35 meropenem, and 68 both imipenem/cilastatin and meropenem, whereas 107 children tolerated meropenem.

As far as non-immediate reactions to β-lactams other than penicillins are concerned, there are only a few large studies and no definitive data on skin-test sensitivity. In a recent study [58], cephalosporins elicited a positive patch-test reaction in 12 (4.1%) of 290 patients with cutaneous adverse reactions to these β-lactams, while meropenem caused positive patch-test reactions in one of two patients. It is interesting to note that only one of the 75 patients with cutaneous eruptions associated with cephalosporins and negative results in allergologic tests reacted to challenges with the suspect cephalosporins (cefadroxil or cephalexin). In another study concerning hypersensitivity reactions to cephalosporins in children [59], 105 subjects with non-immediate reactions were evaluated. Only one child presented positive immediate responses to skin tests with penicillin reagents. Among the 104 children with negative results in allergologic tests, 96 underwent challenges: 95 tolerated them and one reacted to a cefaclor paediatric suspension and tolerated the challenge with a cefaclor capsule.

Drugs Used During Anesthesia, Including Muscle Relaxants

Skin tests are recommended to evaluate patients with immediate reactions during general anesthesia, as well as to reduce the risk of such reactions, identifying patients sensitized to anaesthetic drugs and/or other compounds to be administered during the procedure, and to find safe alternatives [6]. About 60% of hypersensitivity reactions occurring during anesthesia are IgE-mediated, as demonstrated by positive skin tests and/or specific IgE assays [60, 61]. Since 1980, several French studies have recorded the agents responsible for about 4,000 anaphylactic reactions related to anesthesia: muscle relaxants (62%), latex (16.5%), hypnotics (7.4%), antibiotics (4.7%), plasma substitutes (3.6%), and opioids (1.9%) were the most important elicitors [6, 61]. SPTs and IDTs remain the gold standard of diagnosis and should be done 6 weeks after the reaction. All substances used in the anesthesia and perianesthetic period should be tested starting with the highest dilutions (generally, 1/1,000; for muscle relaxants, up to 1/10,000), and then increasing concentrations. As many substances can produce irritative reactions when used undiluted, in order to avoid false positive results, the highest concentrations indicated in Table **6** should not be exceeded. The concentrations recommended by the ENDA concerning IDTs with muscle relaxants have been slightly amended by a large study carried out in healthy volunteers in order to find non-irritating concentrations for such testing [62]. For these drugs, a wheal with a diameter of at least 8 mm and at least twice the diameter of the bleb produced by the injection has been proposed as the criterion for positivity [6]. Succinylcholine appears to pose the greatest risk [61]. An investigation of cross-reactivity with other compounds is encouraged in order to identify reagents which may be tolerated. Considering that premedication does not guarantee that an anaphylaxis will not occur, the only effective preventive measure is to identify the responsible compound by skin tests and serum specific IgE assays, and then completely avoid it.

Iodinated Contrast Media

Immediate- and delayed-reading skin tests with ICM are indicated in patients with severe immediate hypersensitivity reactions and in those with non-immediate skin reactions following administration of ICM, respectively [5, 63, 64]. In a recent European multicenter study [34], 26% and 38% of 220 patients with typical clinical features of immediate and non-immediate reactions to ICM, respectively, presented positive skin tests when undiluted compounds were used for both SPTs and PTs, and ICM diluted 1/10 in 0.9% NaCl were used for IDTs; the specificity of all these tests was higher than 96%. For immediate reactors, the IDTs were the most sensitive tests, whereas delayed-reading IDTs in combination with PTs were needed for optimal sensitivity in non-immediate reactors. In this study [34], cross-reactivity among different compounds was diagnosed in both immediate and non-immediate reactors on the basis of positive responses to skin tests with ICM other than responsible ones. Skin testing with the latter could be useful in finding safe alternatives in ICM-allergic subjects. However, the negative predictive value of skin testing is not yet fully established, as patients with negative skin tests, but positive reactions in provocation tests have been described [65].

Heparins

The most common hypersensitivity reactions to heparins are erythematous plaques, occurring with a delay after subcutaneous application [66], whereas their further development into maculopapular exanthema is seldom observed [67]. Skin and provocation tests are useful diagnostic tools in evaluating hypersensitivity reactions to heparins. In a suspected immediate type hypersensitivity, after a negative SPT with undiluted heparins, an IDT starting at a 1/1,000 dilution is performed. If negative, IDTs are performed with increasing logarithmic concentrations up to a 1/10 dilution. In patients with erythematous plaques and exanthemas, IDTs with 1/10 or undiluted heparins are performed, whereas PTs are

Table 6. Non-Irritative Concentrations of Anaesthetic Agents in Skin Tests (Adapted from Mertes *et al.* [6])

Available Agents		Prick Tests		Intradermal Tests	
Name	**C (mg·mL^{-1})**	**Dilution**	**MC (mg·mL^{-1})**	**Dilution**	**MC(µg·mL^{-1})**
Atracurium	10	1/10	1	1/1000	10
Cis-atracurium	2	undiluted	2	1/100	20
Mivacurium	2	1/10	0.2	1/200*	10
Pancuronium	2	undiluted	2	1/50*	40
Rocuronium	10	undiluted	10	1/200*	50
Suxamethonium	50	1/5	10	1/100*	500
Vecuronium	2	undiluted	2	1/50*	40
Etomidate	2	undiluted	2	1/10	200
Midazolam	5	undiluted	5	1/10	500
Propofol	10	undiluted	10	1/10	1000
Thiopental	25	undiluted	25	1/10	2500
Alfentanil	0.5	undiluted	0.5	1/10	50
Fentanyl	0.05	undiluted	0.05	1/10	5
Morphine	10	1/10	1	1/1000	10
Remifentanil	0.05	undiluted	0.05	1/10	5
Sufentanil	0.005	undiluted	0.005	1/10	0.5
Bupivacaine	2.5	undiluted	2.5	1/10	250
Lidocaine	10	undiluted	10	1/10	1000
Mepivacaine	10	undiluted	10	1/10	1000
Ropivacaine	2	undiluted	2	1/10	200

C = concentration; MC = maximal concentration;
*Concentrations modified according to Mertes *et al.* [62]

less sensitive. In case of negative skin test responses, subcutaneous provocation tests are necessary. In a few cases, additives present in the trade products have been identified as culprit agents [68].

In order to identify alternative compounds, test reagents should include both unfractionated and fractionated heparins, as well as heparinoids and hirudins [67, 69]. In effect, cross-reactivity among heparins is common and may also include heparinoids [67, 69, 70].

Other Skin Test Procedures for Specific Drugs

Non- β-Lactam Antibiotics

Skin testing with parent drugs has proved to be useful for diagnosing IgE-mediated hypersensitivity reactions to many non-β-lactam antibiotics, such as aminoglycosides [71-77], rifampicin [78], vancomycin [79], sulfonamides [80, 81], trimethoprim [82], and teicoplanin [83]. Specifically, there are several reports of single cases of anaphylactic reactions to aminoglycosides, such as streptomycin [72, 74, 75], bacitracin [73], tobramycin [71], and ribostamycin [76], which presented positive skin tests to the culprit drug, generally at concentrations up to 100 mg/ml for prick tests and lower than 10 mg/ml – as little as 0.1 ng/ml, in a case of anaphylactic reaction to streptomycin [74] – for intradermal ones. However, such skin testing in large samples has not been fully validated. In 3 patients with immediate urticarial reaction to rifampicin, intradermal tests with the parent drug at a concentration of 0.006 mg/ml in 0.9% NaCl were truly positive, whereas in *vitro* tests did not contribute to the diagnosis [78]. Anne' *et al.* [79] diagnosed an IgE-mediated hypersensitivity to vancomycin in a patient with an anaphylactic reaction, on the basis of a positive response to an IDT at a concentration of 0.1 µg/ml; in this study, 7 control subjects reacted at concentrations of 10 µg/ml or higher. In a study by Gruchalla and Sullivan [80], skin tests with multivalent sulfamethoxazole-poly-L-tyrosine revealed an IgE-mediated pathogenic mechanism in some patients with immediate reactions to sulfamethoxazole. Shapiro *et al.* [81], evaluating 28 patients with adverse reactions to sulfonamide antibiotics

by skin tests or specific IgE assays with sulfamethoxazole, found that 4 of the 28 who had been skin prick tested and 2 of the 10 who had undergone *in vitro* testing were positive. Alonso *et al.* [82] diagnosed an IgE-mediated hypersensitivity to trimethoprim in 3 subjects on the basis of positive responses to SPTs at concentrations of up to 10 mg/ml in 0.9% NaCl.

In a study by Asero [83], a positive response to an IDT with teicoplanin at a concentration of 75 mg/ml allowed the author to diagnose an IgE-mediated hypersensitivity in a patient who had suffered an anaphylactic reaction to this antibiotic.

On the other hand, skin testing with quinolones and macrolides is not considered a reliable tool in diagnosing hypersensitivity reactions to them [77, 84]. In effect, quinolones and opiates are classical examples of direct histamine-releasing drugs, and the results of skin testing with undiluted compounds have to be interpreted with caution, as irritative positive responses also occur in healthy control subjects [84].

Benahmed *et al.* [85] evaluated 107 patients with adverse reactions to macrolides, performing challenges with the suspected drugs. Thirty-three of the 107 subjects also underwent skin tests with the available injectable macrolides (spiramycin and erythromycin) at concentrations of 10 mg/ml for SPTs and 0.1 to 10 mg/ml for IDTs; at a concentration of 10 mg/ml, the latter were positive in 11 (33.3%) of the 33 subjects: to spiramycin in 8 (4 of whom tolerated challenges) and to erythromycin in 3 (all of whom tolerated challenges).

As far as non-immediate reactions are concerned, in a study by Lammintausta and Kortekangas-Savolainen [58], which evaluated 947 patients with cutaneous adverse drug reactions, clindamycin elicited positive patch-test responses in 12 (19%) of 63 patients, and gentamicin and isoniazid in one of two**.** In a study by Schmid *et al.* [86], patch tests were positive to responsible quinolones (ciprofloxacin, norfloxacin, or moxifloxacin, diluted to 10% or 25% in white petrolatum) in 3 of 6 patients who had experienced exanthemas or AGEP. Delayed-reading positive skin tests have also been reported in patients with maculopapular exanthemas associated with isoniazid, pyrazinamide [87], and pristinamycine [13, 88]. Moreover, a cell-mediated pathogenic mechanism has been demonstrated in some patients with fixed eruptions associated with tetracyclines, cotrimoxazole, or trimethoprim [58, 89, 90], on the basis of positive responses to patch tests applied on the previously involved site.

Nonsteroidal Anti-Inflammatory Drugs

Although most cutaneous reactions to nonsteroidal anti-inflammatory drugs (NSAIDs) appear to be induced by a non-allergic hypersensitivity pathogenic mechanism, in many immediate reactions to pyrazolones, an IgE-mediated hypersensitivity has been diagnosed by skin testing [91, 92]. Kowalski *et al.* [91] diagnosed such hypersensitivity, on the basis of positive responses to IDTs with 0.001% and 0.01% solutions of noraminophenazone, in 11 of 23 patients who underwent these tests. In a study by Himly *et al.* [92], 44 of 53 patients were positive to skin tests with propyphenazone at a concentration of 0.5%.

On the other hand, in some non-immediate reactions to NSAIDS, such as pyrazolones, diclofenac, piroxicam, and acetaminophen, a cell-mediated pathogenic mechanism may be involved and delayed-reading IDTs and PTs are useful in assessing such reactions [14, 58, 93-95].

Chemotherapy Agents

Skin testing with platinum salts has proved to be a very useful tool in assessing allergic hypersensitivity to these drugs [96, 97]. Leguy-Seguin *et al.* [96] diagnosed by skin testing an IgE-mediated hypersensitivity reactions to platinum salts, such as cisplatin, carboplatin, and oxilaplatin, in 21 patients. SPTs with pure injectable compounds were positive in 5 subjects, IDTs at concentrations of up to 1 mg/ml were positive in 12 (always when reactions had occurred less than 2 hours after infusions), and delayed-reading of skin tests in 3, whereas PTs were negative in all the 21 patients tested. In a recent study by Castells *et al.* concerning rapid desensitization protocols with chemotherapic drugs [97], 60 patients underwent skin test with carboplatin at concentrations of up to 10 mg/ml, and 53 (88%) had positive results. Skin testing with platinum salts has also been used to identify patients at risk for reactions, with a negative predictive value of 96% [98].

In a study by Billet *et al.* [99], the incidence of allergic reactions to intramuscular *Escherichia coli* asparaginase was 24.8% (31 of 125 patients). Intradermal testing has generally been performed before initial administration of asparaginase to test for immunologic hypersensitivity [100].

Insulins

Reactions to insulin therapy are predominantly local, at the injection site; however, systemic reactions have also been described, not only to porcine and bovine insulin, but also to the recombinant human one [101, 102]. Skin tests have been employed in order to identify allergic hypersensitivity reactions, assess any cross-reactivity among different compounds, and find safe alternatives. Reactions to protamine-containing insulins may be caused by the protamine component (added in order to delay absorption) in the insulin preparation. Dykewicz *et al.* [103] described two patients who had experienced an anaphylactic reaction to the protamine insulin, but who tolerated regular insulin, which was found to be negative in skin testing.

Anticonvulsants

Anticonvulsant or antiepileptic drugs, particularly aromatic ones (phenytoin, carbamazepine, oxcarbazepine, and phenobarbital), can provoke cutaneous eruptions, as well as a severe DRESS [13, 104, 105].

PTs and, to a lesser extent, delayed-reading IDTs can be useful tools for diagnosing such hypersensitivity reactions. With regard to carbamazepine, the percentage of positive responses to patch tests can be as high as 69.2% when its metabolites are also used in patch testing [106]. In many studies, different carbamazepine concentrations (ranging from 1% to pure powder) in different vehicles (petrolatum, distilled water, ethanol) have been used and positive reactions have been seen at all concentrations [105]. However, a severe systemic exfoliative eruption after patch testing with crushed 200-mg carbamazepine tablets has been reported

[107]. Thus, percentages of carbamazepine up to 20% weight/weight in white petrolatum seem to be sufficient to induce positive patch-test reactions and could also be recommended in order to avoid the risk of systemic reactions. On the other hand, some weak reactions may be missed.

With regard to hypersensitivity reactions to anticonvulsants other than carbamazepine, Osawa *et al.* [108] patch tested 23 subjects with cutaneous eruptions associated with anticonvulsant therapy: 6 of them had reacted to carbamazepine, 10 to phenobarbital, 5 to sodium valproate, and 2 to phenytoin. Carbamazepine was tested at a concentration of 1% in white petrolatum, phenobarbital at 1% and 20%, and sodium valproate at 1% and 10%. A total of 13 (56.5%) out of 23 subjects displayed positive responses to patch tests; specifically, 4 of 6 to carbamazepine, 4 of 10 to phenobarbital, 4 of 5 to sodium valproate, and 1 of 2 to phenytoin. In this study [108], 9 patients with adverse reactions to phenobarbital and 1 to phenytoin were also evaluated by delayed-reading intradermal tests. It is interesting to note that intradermal-test sensitivity was lower than that of patch tests.

Corticosteroids

Corticosteroids may elicit immediate and non-immediate hypersensitivity reactions, which can be diagnosed on the basis of positive SPTs, IDTs and/or PTs, with considerable cross-reactivity among different compounds [109-112]. In evaluating allergic contact dermatitis to corticosteroids, the sensitivity of patch testing (especially if testing is performed with an extended series of compounds) is very good [113]. On the contrary, in case of non-immediate hypersensitivity reactions to systemic corticosteroids, the allergologic test sensitivity is low. In a study by Padial *et al.* [114], only 2 of the 38 patients with non-immediate reactions, such as delayed-appearing urticaria and maculopapular exanthema, displayed positive delayed-reading intradermal tests and patch tests to the responsible drugs, while 21 of the 32 patients who agreed to undergo challenges reacted to them.

Miscellanea

A cell-mediated hypersensitivity mechanism has been demonstrated on the basis of positive responses to patch tests and/or delayed-reading intradermal tests in patients who developed delayed hypersensitivity reactions, mainly maculopapular rashes, to drugs such as practolol, diazepam, acyclovir, hydroxyzine, tetrazepam, diltiazem, captopril, pseudoephedrine, stepronin, and abacavir [13, 14, 58, 115-119].

With regard to immediate reactions, administration of intravenous streptokinase for treatment of acute myocardial infarction carries the risk of anaphylaxis. Because of its specificity and sensitivity, skin testing immediately before streptokinase administration has been proposed for identifying patients at risk for immediate-type allergic reactions to this drug [120]. Similarly, performing SPTs before administration of chymopapain has been proposed to prevent anaphylaxis in chemonucleolysis candidates [121].

CONCLUSIONS

Skin tests together with the history are the most readily available and useful diagnostic tools in evaluating drug hypersensitivity reactions. In many cases, skin tests allow the physician to avoid hazardous provocation tests. Skin tests have also been employed in order to identify patients at risk.

Whereas for β-lactam antibiotics, muscle relaxants, and ICM, the value of skin tests has been proven in a large number of patients and standardized protocols exist, for many other drugs this still has to be addressed in multicenter studies with common protocols.

In any case, much research needs to be done in order to standardize both PTs and IDTs (particularly those performed with non-injectable drugs), improve their sensitivity, and establish their negative predictive value.

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