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Patch Testing for the Diagnosis of Anticonvulsant Hypersensitivity Syndrome

A Systematic Review

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Abstract

Anticonvulsant hypersensitivity syndrome (AHS), also known by the other names drug rash (reaction) with eosinophilia and systemic symptoms (DRESS) and drug-induced hypersensitivity syndrome (DIHS), is a rare and potentially fatal reaction that occurs in susceptible patients after exposure to

certain drugs, including aromatic anticonvulsants. Because of its ill-defined clinical picture and resemblance to other diseases, the diagnosis of AHS is often difficult and requires a safe and reliable diagnostic test. The skin patch test has been proven to be very useful for prediction and diagnosis of some types of hypersensitivity reactions such as delayed drug eruptions to β-lactam antibacterials. However, the diagnostic value of patch testing for AHS is yet to be determined and its negative predictive values (NPVs) and positive predictive values (PPVs) are still unknown.

This systematic review attempts to evaluate the usefulness of patch tests in the diagnosis of AHS and to examine different technical aspects of patch testing that may contribute to its performance. We included studies in which aromatic anticonvulsant drugs are the likely causes of the hypersensitivity reaction.

Analysis of original publications from 1950 to August 2008 and cited in PubMed, MEDLINE and EMBASE has revealed contradictory findings, possibly due mainly to the use of unstandardized methods. Numerous factors have been suggested to affect the final result of the test, including the following: type of drug tested; concentration of drug and vehicle used; timing of the test after exposure; and the clinical picture of the reaction. The PPV of the test in optimal conditions was as high as 80–90% depending on the drug tested. On the other hand, this value is around 10–20% in many other published studies.

Although patch testing may be a useful diagnostic test for AHS, accurate determination of its sensitivity and specificity is yet to be achievable due to the lack of a gold standard test against which the performance of patch testing can be measured. Its PPV appears to be higher than its NPV, a matter that necessitates the use of other confirmatory tests in case of negative patch tests (e.g. careful systemic rechallenge). The benefit of testing appears to be maximal with certain drugs (i.e. carbamazepine and phenytoin) and for specific clinical manifestations (strong reactions). It should be performed 2–6 months after recovery from the date of the ADR for best results, with adequate vehicle control.

Adverse drug reactions (ADRs) have been defined as undesirable effects associated with the therapeutic use of drugs.[1] An ADR is defined by the WHO as a noxious and unintended response to a drug that occurs at a dose normally used in man for prophylaxis, diagnosis or therapy.[2] ADRs represent a major health problem worldwide with high rates of morbidity and mortality.[3-6] Lazarou and colleagues[4] have estimated in a meta-analysis that ADRs were responsible for nearly 100 000 deaths in the US in 1994. Despite the fact that this study has been criticized,[7] it does lend credence to the seriousness of this problem. Indeed, the authors of this study have estimated that ADRs are ranked between the forth and sixth leading cause of death, after heart disease, cancer, stroke, pulmonary disease and accident, in the US and Canada. It has also been demonstrated that drug-related injuries occur in at least 7% of hospitalized patients,[4] although accurate estimation of such cases is difficult due to under-reporting.[8] In addition, ADRs also represent a serious economic burden on the healthcare system.[9]

ADRs have been classified into the following two types: type A reactions, which are usually predictable, dose-dependent and related to the pharmacological action of the drug; and type B reactions, which are unpredictable, have a delayed onset and cannot be explained by the

pharmacological action of the drug.[10] Type B reactions are typically dose-independent; however, dose-dependence of these type of drug reactions can exist at higher dose ranges than conventional pharmacological dose-response relationships.[11] Type B ADRs or idiosyncratic reactions comprise various types of reactions, such as immune-mediated (allergic, immunological reactions), which include drug hypersensitivity reactions or drug hypersensitivity syndrome (DHS), and non-immune-mediated (sometimes called metabolic idiosyncrasy).[12] Gell and Coombs[13] classified immune-mediated reactions into four types: type I reactions (immunoglobulin E-mediated); type II reactions (through cytotoxic mechanisms); type III reactions (immune complex-mediated); and type IV reactions, which involve activation of T cells and are known as 'delayed hypersensitivity'. Type IV reactions have recently been subdivided according to the heterogeneity of T-cell function into types IVa, IVb, IVc and IVd.[14,15] Although an elegant and mechanism-based classification system, many serious and probable immune-mediated ADRs do not fit into these established categories.[16] DHS is thought to belong to type IV, T-cell-mediated delayed reactions.[17]

DHS is a rare but potentially lethal host-dependent ADR that occurs in susceptible patients upon exposure to specific agents. It has been estimated that idiosyncratic reactions, of which DHS represents a major component (around 10%), constitute from 3% to 25% of all ADRs.[18] Because of its unpredictable nature and potential severe morbidity and mortality, DHS is a major problem for patients, clinicians, drug regulators and the pharmaceutical industry and often deprives patients of effective therapy.

The nomenclature of this type of drug hypersensitivity reaction has long been a topic of debate.[19,20] Delantin hypersensitivity syndrome, sulfone syndrome, dapsone hypersensitivity syndrome, allopurinol hypersensitivity syndrome, drug-induced delayed multiorgan hypersensitivity syndrome (DIDMOHS), anticonvulsant hypersensitivity syndrome (AHS), drug rash (reaction) with eosinophilia and systemic symptoms (DRESS) and drug-induced hypersensitivity syndrome (DIHS) have all been suggested as names and acronyms for this disorder.[20,21] Although no consensus has emerged thus far, the last three are the most widely used terms. However, for the purpose of this review, it was felt that AHS is the most relevant term because only reactions related to aromatic anticonvulsant drugs (ACDs) were reviewed.

The objective of this systematic review was to critically review all the relevant publications related to the use of the patch test in the diagnosis of AHS. We also aimed at discussing the technical aspects of this *in vivo* test that contribute to its performance.

1. Anticonvulsant Hypersensitivity Syndrome (AHS)

Aromatic ACDs such as phenytoin, carbamazepine and phenobarbital (phenobarbitone) as well as some newer agents, including lamotrigine, oxcarbazepine, felbamate and zonisamide (figure 1), have been implicated in eliciting a whole repertoire of hypersensitivity reactions ranging from simple maculopapular skin eruptions to a severe life-threatening disorder. Upon exposure to an implicated drug, a constellation of symptoms develop, including fever, skin eruption and internal organ dysfunction.[22-33] Implicated drugs include aromatic anticonvulsants (carbamazepine, phenytoin, phenobarbital, lamotrigine), sulfonamide antibacterials, dapsone, minocycline, terbinafine, azathioprine and allopurinol.[34] Although AHS is typically defined by the triad of symptoms (i.e. fever, skin rash and internal organ involvement), it is quite difficult to associate a typical clinical picture to this syndrome as AHS can manifest as a wide range of clinical symptoms. Affected patients may develop fever, a skin eruption (from a mild skin rash to severe eruptions such as Stevens-Johnson syndrome [SJS] and toxic epidermal necrolysis [TEN]), and internal organ involvement (either asymptomatic or symptomatic).[1,24,35] The multivisceral involvement of this illness may include blood dyscrasias (e.g. eosinophilia, thrombocytopenia), hepatitis, nephritis, myocarditis, thyroiditis, interstitial pneumonitis and encephalitis. Other clinical

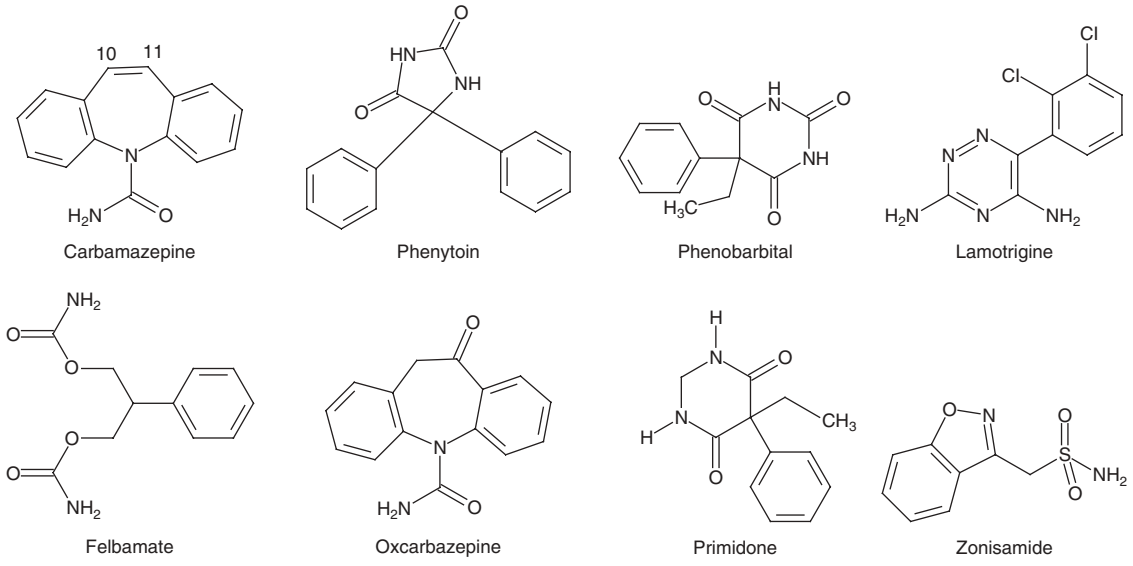

Fig. 1. Chemical structures of aromatic anticonvulsant drugs.

features of AHS are facial oedema, tonsillitis, pharyngitis, mouth and lip ulcers, enlargement of liver and spleen, myopathy and disseminated intravascular coagulation.[35-39] It has been estimated that the incidence of AHS lies between 1 in 1000 to 1 in 10 000 among patients chronically treated with phenytoin and carbamazepine.[40] However, these incidences are believed to be inaccurate as a result of under-reporting.[41]

The exact molecular mechanisms involved in AHS are not well understood. In fact, it is thought that multiple mechanisms are involved, sometimes simultaneously, to produce a single event.[37,42] Discussing detailed molecular mechanisms underlying AHS is beyond the scope of this review; nonetheless, some recent comprehensive reviews on this subject are available.[37,43,44] In general, AHS is believed to be immune-mediated in all cases,[17,45] and the generation of reactive electrophilic drug metabolites that react selectively and non-enzymatically at nucleophilic sites on multiple proteins to form immunogenic drug metabolite-protein adducts is proposed to be the initial mechanistic step in the cascade of cell-based reactions that results in the clinical symptoms.[22,46-48] At least a few of the proteins that are covalently modified by metabolites of drugs causing AHS are likely to be involved in eliciting the immune response that characterizes these hypersensitivity reactions.[37,47,49]

2. Diagnosis of AHS

A validated, gold standard *in vitro* test for diagnosis or prediction of AHS is not yet available. In fact, the value of all currently used *in vivo* and *in vitro* tests is widely controversial and their sensitivities, specificities and variability are yet to be determined.[50-54] Currently, the diagnosis of AHS is based on clinical expertise that is comprised of (i) a thorough clinical history, including detailed medication history; (ii) a comprehensive physical examination; and (iii) available laboratory data. Misdiagnosis of AHS is very common because the syndrome resembles other conditions such as infections, collagen vascular disorders and haematological/oncological conditions.[25,37] An *in vivo* systemic rechallenge (drug provocation testing or controlled re-exposure) is considered to be the gold standard in AHS diagnosis,[55] although ethically this is highly contentious, as a rechallenge with the implicated drug may result in severe morbidity or even death. Presently, there are at least three tests available for diagnosis of AHS, namely

the patch test, the lymphocyte transformation test (LTT) and the lymphocyte toxicity assay (LTA).[50,56-58] The use of the patch test for the diagnosis of AHS is reviewed here.

3. Research Methodology

We performed the systematic literature search using the databases PubMed, EMBASE and MEDLINE from their commencement to the 4th week of August 2008 (figure 2).

3.1 Search Strategies

The first search (search strategy I) was carried out using key words 'anticonvulsant' and 'antiepileptic' in their singular, plural and truncated forms. These terms were also mapped to their medical subject headings (MeSH) terms. We also searched for individual aromatic ACDs, including 'carbamazepine', 'phenytoin', 'phenobarbital', 'oxcarbazepine', 'primidone', 'lamotrigine', 'felbamate' and 'zonisamide' both as key words and as MeSH terms when available and the option 'explode' was used. The obtained results were combined using 'OR'.

In addition, a second search (search strategy II) was carried out using the key words 'skin test', 'patch test' and 'epicutaneous test' in their singular, plural and truncated forms. These terms were also mapped to their MeSH terms when available and the option 'explode' was used.

The results of the first and second searches were then combined using 'AND'. The search results were then limited to original articles that were published in the English language and performed on human subjects. At this point, we retrieved 244 articles from PubMed, 163 articles from MEDLINE and 184 articles from EMBASE. These publications were then manually reviewed and the following selection criteria were applied: (i) original articles; (ii) used patch testing for the purpose of diagnosis of suspected AHS as a result of one or more aromatic ACD(s); and (iii) contained sufficient technical data.

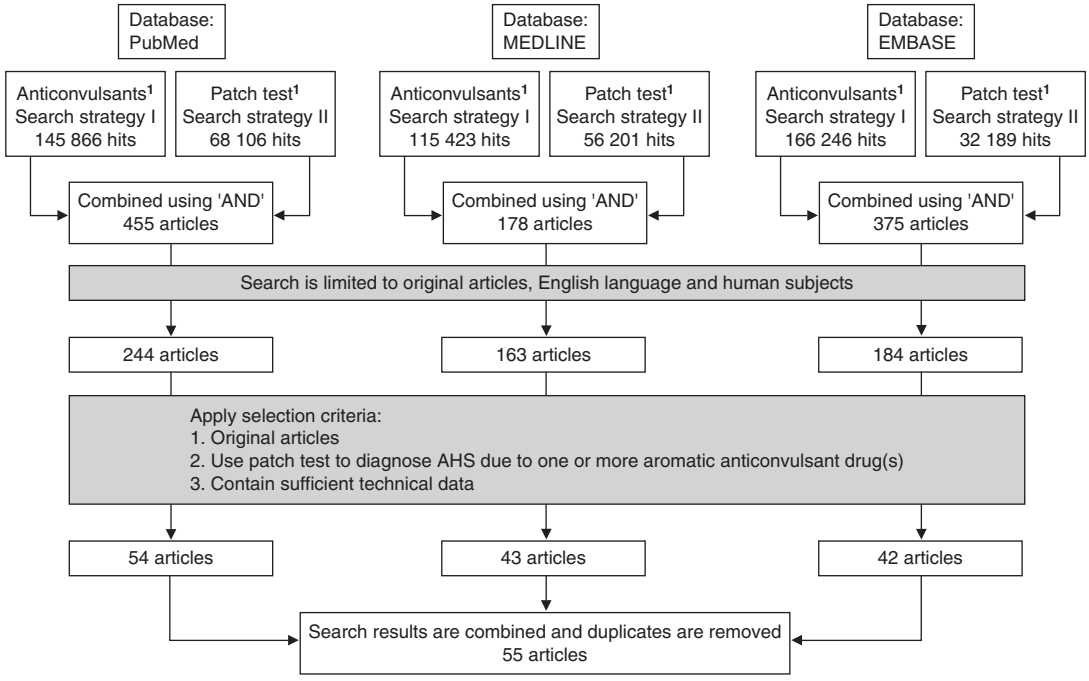

Fig. 2. Flow chart of literature search and retrieval process. **1** Search strategies 'anticonvulsants' and 'patch test' include all relevant medical subject headings and key words as described in the Research Methodology section. **AHS** = anticonvulsant hypersensitivity syndrome.

Applying our selection criteria, 54 articles from PubMed, 43 articles from MEDLINE and 42 from EMBASE were found to meet our selection criteria. The search results from the three databases were then combined and duplicates were removed. The final number of included articles from the three databases was 55.

4. Patch Tests in the Diagnosis of AHS

Patch testing utilizes the concept that a localized, confined, immune-mediated reaction to the agent of concern can be reproduced by introducing the agent through the skin. Briefly, the patch test is performed by applying the drug to be tested (ground commercially available tablets, liquid forms or pure drug powder) on the skin (usually of the upper back) using different devices that give standard surface area exposure. One widely used device is the Finn chamber. The drug is diluted in suitable media (usually petrolatum, water or ethanol) and the media alone is used as control. The test is then read for appearance of local reaction after different time periods (20 minutes to 4 days).[59]

This concept has been proven and extensively used for contact irritants and systemically administered drugs such as the β-lactam antibacterials.[60,61] Presently, the positive predictive value (PPV) and the negative predicative value (NPV) of the patch test in the diagnosis of AHS remain to be determined and its real value is still unknown.[50] The percentage of concordance between clinically suspected hypersensitivity reactions and positivity of patch testing varies considerably because of lack of test standardization.[42,53,62]

5. Determinants Affecting Patch Test Results

5.1 Epicutaneous Penetration

An important determinant of patch test success is the ability of the tested agent (drug) to cross the skin (epicutaneous penetration) and come into contact with the processing cells of the immune system (presumably dendritic cells).[63] This property depends largely on the physicochemical characteristics of the drug to be tested, its concentration/formulation and the vehicle in which the drug is dispersed or solubilized. The physiochemical characteristics of the drug determine its polarity and lipid-solubility, thus affecting the ability of the drug to cross the skin barrier and reach the target cells.[64] In this regard, either the drug itself or its metabolite can be used, although many reactive metabolites are not available commercially due to their instability, and purity of the reactive metabolites tested in this manner is an issue. In addition, reactive metabolites may not be able to cross the epithelial barrier as they tend to be less lipophilic and, in some cases, the reactive metabolite is unknown. Unfortunately, it is not yet possible to comment on the benefit of using metabolites of ACDs in patch testing because of the paucity of literature on this subject.[51,62] There are also cases where opposing results have been obtained when patch testing a drug and its main metabolite in the same patient.[62,65] Surveying the literature, it seems that the ACDs that are most commonly involved in eliciting AHS are carbamazepine and, to some degree phenytoin. This may be because of frequency of use of carbamazepine and phenytoin as opposed to prevalence of AHS; therefore, it is not surprising to find many more investigators interested in studying the toxicity of these drugs compared with other ACDs. Another possible reason for choosing to work with carbamazepine is because it is easier to work with in regard to the frequency of positive results in highly imputable cases.

Once absorbed, carbamazepine is initially metabolized in the liver (or skin) via cytochrome P450 (CYP) 3A4 and CYP2C8, into at least 33 different metabolites.[66-69] One of the main metabolites, which is also known to have pharmacological activity, is carbamazepine 10,11 epoxide, which is stable and available commercially for research purposes. Lee et al.[62] patch tested both carbamazepine and carbamazepine 10,11 epoxide on 13 patients who had exhibited a skin reaction to carbamazepine manifested as a maculopapular cutaneous eruption. Seven of the 13 patients gave positive patch tests with the parent drug but negative results with the metabolite; two reacted only to the metabolite and one patient tested positive to both agents. In the same study, all 39 control subjects who were taking

antiepileptic drugs, including carbamazepine, gave negative patch test results to both carbamazepine and carbamazepine 10,11 epoxide. Although the PPV of the patch test for carbamazepine in this study was good (61.5%), the low percentage of positive tests when using carbamazepine 10,11 epoxide (23.0%) is difficult to explain. The authors interpreted these results to be due to either the low concentration of carbamazepine 10,11 epoxide used or to efficient metabolism of carbamazepine 10,11 epoxide, for example by epoxide hydrolase, in some of the patients. The latter explanation is more likely.

The use of a reactive metabolite in patch testing has always been hindered by lack of knowledge of the role of each metabolite of a drug in eliciting hypersensitivity reactions and response to the exact testing procedure as well as lack of availability of most of the suspected metabolites due to their chemical instability. Duhra and Foulds[66] patch tested carbamazepine and oxcarbazepine as well as some of their metabolites (but not carbamazepine 10,11 epoxide) in a patient with suspected carbamazepine hypersensitivity. Only carbamazepine gave positive patch test results and they suggested that the double-bond between positions 10 and 11 of the azepine ring (figure 1) is critical for skin reactivity. No other study is available in the published literature using patch testing with metabolites of ACDs.

5.2 Type of Drug Tested

It has been shown that the predictive value of a patch test depends largely on the type of drug implicated in the ADR.[59] Galindo et al.[70] have investigated 23 different types of ADR, including generalized rash, fever, arthralgia, lymphadenopathy, palpable purpura, facial erythema, angioedema and erythema multiforme, developed to ACDs in 15 patients using patch testing. They found the patch test to be most useful for ADRs involving carbamazepine (PPV 75%) and phenytoin (PPV 60%), whereas the rate for a positive test was very low (25%) with phenobarbital and lamotrigine. The good PPV observed with carbamazepine does not seem to be affected by the vehicle used, i.e. whether it is liquid (water or ethanol) or semisolid (petrolatum).[31,71] One explanation for the good PPV of patch testing with carbamazepine could be its high lipophilicity, which may facilitate its percutaneous penetration and intracellular movement during patch testing. Indeed, carbamazepine has very good lipophilic properties and a log K_{oct} (N-octanol/water partition coefficient) value of 2.7, which is near the optimum value of 2.5 for transdermal permeation, although other parameters can be enhanced through some modifications to the chemical structure of the compound.[72]

5.3 Concentration of Tested Drug

The ideal drug concentration in patch testing of anticonvulsants is critical in obtaining positive results in affected patients without inducing non-specific local irritation, which may be falsely interpreted as positive results.[60] The concentration selected should give negative results in control subjects.[73] Because the exposed surface area of the skin is standard (e.g. using Finn chambers) the amount of drug used is always expressed as concentration (weight by volume). In published data, the drug concentration used with ACDs ranged from 0.0001% to 100% pure substance, but the most commonly used concentrations were between 1% and 10%. However, 0.1% has been the lowest reported concentration at which a positive patch test to carbamazepine was observed.[74-76]

It has been recommended to use pure drug, whenever available, in order to avoid false-positive results due to hidden additives in the drug formulations,[77] degradation products or impurities. In all cases, certain guidelines for the preparation of commercially available drug formulae for patch testing have been suggested.[59,73]

5.4 Vehicle

Petrolatum has been a preferred medium for patch testing of skin sensitizers because it gives good occlusion and prevents drug degradation as a result of hydrolysis.[78] Its use has yielded satisfactory results with patch testing of ACDs,[31,33,62,64,74-76,79-86] although, other liquid solvents, such as water,[87] saline,[81] ethanol,[88] methanol,[89] acetone[90] and propylene glycol,[91]

have also been used. Nonetheless, it appears that using different vehicles does not alter the results,[71] although some liquid vehicles evaporate during the test, possibly affecting the concentration at which the drug is introduced.

In addition, applying control patches of the vehicle at the same time as the drug is critical because some patients may be sensitive to the vehicle itself, especially if it is not of high purity.[92] The state of the drug in aqueous vehicle or in a semisolid medium, such as petrolatum, are different since the compound may dissolve in the liquid vehicle but be dispersed as undissolved crystals in the semisolid medium. Thus, we might expect to have better delivery of the drug using the liquid vehicle rather than petrolatum. In fact, using *in vitro* mounted human skin and chromate preparations as a model, Gammelgaard et al.[93] demonstrated a better skin permeation of the chemical (potassium dichromate) with an aqueous vehicle. It is also interesting to note that paracetamol (acetaminophen) gave a positive patch test when using an aqueous vehicle and negative patch test when petrolatum was used as the vehicle.[33]

5.5 Timing

Another factor that seems to be critical to the final result of patch testing is the timing of the test in regard to the beginning of the hypersensitivity reaction. Some authors[94] have recommended performing the patch test within 6 months following the reaction to avoid false-negative results because it is not known how long drug reactivity lasts. However, others have recorded positive patch tests 6 months to 2 years after the reaction.[76,84,86,87,95,96] In fact, positive patch test results have been obtained in patients tested 12 years after the adverse reaction to drugs such as sulfamethoxazole.[97] This may not be surprising as drug-specific T cells can be detected for decades following an adverse reaction.[53] It is not known if this phenomenon of long-lasting drug reactivity is drug-dependent, although the frequency of drug-specific T cells is apparently drug-dependent.[97]

On the other hand, Jones and co-workers[84] have reported false-negative patch test results to carbamazepine when the test was performed during or right after the hypersensitivity episode. In contrast, others[31] have warned about false-positive patch test results if the test is performed during the increased reactivity period of the hypersensitivity reaction and recommend waiting for at least 2 months after the subsidence of the reaction before performing the test. However, positive patch test results have been obtained when the test was performed during or right after recovery from the reaction.[33,71,81,85,90,98-100]

In reviewing different studies, it seems clear that performing patch tests during the acute phase of the reaction appears to yield low rates of positive results,[88,100-102] and the optimal timing for the test in this regard appears to be between 2 and 6 months after the reaction. No mechanistic explanation is available as to why the reaction is not detectable early on; however, some have speculated that transient immune depression during the reaction produces this refractory period.[101] Others propose that transient selective recruitment of antigen-specific lymphocytes into target organs may lead to the low number of such cells in the peripheral blood, and thus low reactivity.[88] However, in the case reported by Okuyama et al.,[101] other factors may have contributed to the negative results of the patch tests for carbamazepine hypersensitivity during and immediately after the reaction, including topical and oral co-administration of steroids during the illness. This observation is supported by the appearance of slightly positive LTTs during the early stages of the reaction.

5.6 Clinical Picture

The clinical picture of the AHS seems to correlate with patch test results, in that patients with certain types of clinical manifestations seem to react differently to the test. This is because the clinical manifestations reflect the underlying and integrated immunological mechanisms of the reactions, which probably differ in one or more aspects from patient to patient and from one drug to another in individuals.[102,103] Some of these underlying reactions are unlikely to be recognized by patch testing, or may not involve the

immunological mechanisms that the patch test was designed to detect. For instance, when the patch test was used on patients who developed different types of cutaneous ADRs, such as exanthemas, fixed drug eruptions or urticaria, positive results were more frequently observed with patients with exanthema than in patients with other types of cutaneous ADRs.[50]

Similarly, Alanko[71] studied 18 patients with different forms of cutaneous reactions to carbamazepine. Of these, 15 were confirmed by oral rechallenge. Patients with maculopapular exanthematous eruptions, exfoliative erythrodermas or erythema multiforme were found to give positive patch test results in about 70% of those tested, whereas those with other types of skin reactions, including fixed drug eruptions, urticaria and other types of exanthema, all had negative patch test results. However, Alanko et al.[104] could demonstrate positive patch test results in patients with fixed drug eruption only if the test were performed on the site of an old lesion and not on unaffected skin. Similarly, Galindo and co-workers[70] have also suggested a correlation between the histological features of the hypersensitivity reaction and the predictability of testing such as patch tests. Puig et al.[74] reported that the clinical type of ADR plays a critical role in the sensitivity of the patch test, which appears to be maximal for maculopapular or morbilliform reactions.

Of particular importance, delayed hypersensitivity reactions may take more than one cutaneous form, even in the same patient.[53,105] Cutaneous manifestations of reactions to ACDs come in many different forms,[24] some of which could be of a pseudo-allergic nature,[106,107] i.e. they may not be mediated by the usual immune mechanisms. Those reactions, although they mimic true allergic reactions, are unlikely to be detectable by the patch test.[53] This may explain the low rate of positive patch test results on AHS patients reported by some investigators.[50,80,83,51,100]

5.7 Other Factors

Other factors that may affect the outcome of the patch test in general are age, sex and ethnic origin of the patient. Many parameters of skin function, such as thickness, pH, blood flow and content of lipid, water and protein, are known to change during aging.[108-111] These changes can affect the ability of the applied drug to penetrate the skin and elicit its effects. With contact allergy, contradictory literature reports have appeared regarding the effect of age, sex and ethnic origin on results of the patch test.[109,112,113] However, these factors have not been evaluated directly in patch testing of ACDs, and further comprehensive work is essential if the contributions of these factors to the variability in patch test results are to be completely understood (table I).

6. Discussion

Our systematic review reveals that there is a deficiency in large-scale studies determining the usefulness of patch testing in the diagnosis of AHS. Lammintausta and Kortekangas-Savolainen[50] performed a retrospective study analysing the results of skin tests including patch testing, performed on 947 patients with suspected cutaneous ADRs during a 13-year period, of whom 56 had been exposed to ACDs. Tested patients had developed a wide range of cutaneous symptoms, including exanthema, urticaria, angio-oedema, fixed drug eruption, vasculitis, purpura and erythema multiforme. Unfortunately, the percentage of positive tests among these patients was lower than 20% and no oral rechallenge was performed to validate the predictive value of the patch test in such cases.

In another study to investigate the suitability of the patch test or the LTT to detect carbamazepine allergy, Troost and colleagues[88] tested a number of patients using both techniques. Correlation between positive results of both tests was rather low ($r = 0.39$; $p = 0.0022$). Among a total of 59 patients displaying adverse effects to carbamazepine, 23 had positive LTTs and only 8 of the 23 LTT-positive patients had a positive patch test (35%).

Among the published studies, the PPV of the patch testing seems to depend on the type of antiepileptic drug under investigation, with the highest values obtained with carbamazepine and

Table I. Summary of data: use of patch testing to investigate anticonvulsant hypersensitivity syndrome

Type of study	No. of pts	No. of controls	Drug[a]	Conc. [w/v] (% unless otherwise indicated)	Vehicle	Time[b]	Frequency of positive result (%)	Reference
Case report	1	10	Phenytoin	1, 5	Sal	6 mo	1/1	
	1	10	Carbamazepine	0.1–20	Sal	6 mo	0/1	
	1	10	Phenobarbital (phenobarbitone)	10–20	Sal	6 mo	0/1	114
Case series	1	0	Carbamazepine	0.1–10	NA	4 wk	1/1	115
Case series	1	0	Phenytoin	1	Wat	DUR	1/1	
	4	0	Carbamazepine	5	Petr	DUR	3/4 (75)	33
Case series	8	34	Carbamazepine	5–20	Petr	1–120 mo	6/8 (75)	
	1	34	Phenobarbital	5–20	Petr	1–120 mo	1/1	
	1	34	Oxcarbazepine	5–20	Petr	1–120 mo	1/1	
	1	34	Valproic acid	15–60	Petr	1–120 mo	1/1	116
Case report	1	0	Phenytoin	1, 10	Petr/wat	3 mo	1/1[c]	
	1	0	Carbamazepine	1, 10	Petr/wat	3 mo	1/1[c]	31
Case report	1	0	Carbamazepine	NA	NA	NA	1/1	117
Retr. cohort	37	5	Carbamazepine	1–30 μg/mL	Petr/sal/eth	2 mo–20 y	7/37 (18.9)	
	6	5	Phenytoin	1–30 μg/mL	Petr/sal/eth	2 mo–20 y	2/6 (33.3)	
	8	5	Oxcarbazepine	1–30 μg/mL	Petr/sal/eth	2 mo–20 y	1/8 (12.5)	
	5	5	Lamotrigine	1–30 μg/mL	Petr/sal/eth	2 mo–20 y	0/5 (0)	50
Case report	1	3	Phenytoin	50 mg/mL	NA	2 mo	1/1	118
Case report	1	10	Carbamazepine	10	Petr	NA	1/1	79
Case series	1	10	Phenytoin	12.5	PBS	NA	1/1	
	1	10	Carbamazepine	20	PBS	NA	1/1	
	1	10	Oxcarbazepine	12.5	PBS	NA	1/1	119
Case series	10	40	Phenytoin	10	Petr/eth	NA	3/10 (30)	80
Case report	1	3	Valproic acid	Pure	Pure	3 mo	1/1	120
Case report	13	39	Carbamazepine	10	Petr	NA	7/13 (53.8)	
	13	39	Carbamazepine 10,11 epoxide	1 μg/mL	Eth	NA	3/13 (23)	62
Case series	8	20	Carbamazepine	5	Wat	>2 mo	5/8 (62.5)	
	5	20	Phenytoin	5	Petr	>2 mo	3/5 (60)	
	4	20	Phenobarbital	5	Petr	>2 mo	1/4 (25)	70

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Table I. Contd

Type of study	No. of pts	No. of controls	Drug[a]	Conc. [w/v] (% unless otherwise indicated)	Vehicle	Time[b]	Frequency of positive result (%)	Reference
Case report	1	5	Lamotrigine	10	Petr	DUR	1/1	98
Case report	1	0	Carbamazepine	NA	NA	1 wk	0/1	70
Cohort study	1	20	Carbamazepine	400 μg/mL	PBS	6–8 wk	0/1	121
Case report	1	0	Carbamazepine	0.1, 1, 2	Petr	1–2 wk	1/1	122
Case report	1	0	Lamotrigine	50	Petr	2 d	1/1	123
Case report	1	0	Carbamazepine	5	Petr/sal	Aft. rec.	1/1	81
Case report	1	0	Valproic acid	20	Wat	9 mo	1/1	87
Case report	1	15	Carbamazepine	1, 5	Petr	5 mo	1/1	82
Case series	2	0	Carbamazepine	1, 5	Petr	NA	1/2	124
Case report	1	20	Phenytoin	1–20	Petr/wat	2 mo	1/1	125
Case series	20	0	Carbamazepine, phenytoin, phenobarbital	10	NA	NA	12/20 (60)	34
Case series	4	5	Carbamazepine	1, 10	Petr	>1 mo	4/4 (100)	126
Case report	1	0	Carbamazepine	0.1–10	Petr/wat	3 mo	0/1	127
Case series	11	20	Carbamazepine, phenobarbital	1	Petr	3–8 wk	5/11(45.5)	83
Case report	1	0	Carbamazepine, phenytoin, oxcarbazepine	10	Eth	2 mo	1/1	99
Case series	61	11	Carbamazepine	10	Eth	DUR	12/61 (20)	
	59	11	Oxcarbazepine	10	Eth	DUR	8/59 (14)	88
Case series	7	40	Carbamazepine	1, 5, 10	Petr	>1 mo	6/7 (85.7)	74
Case report	1	10	Phenytoin	1	Petr	NA	1/1	
	1	10	Carbamazepine	1	Petr	NA	1/1	
	1	10	Phenobarbital	5	Petr	NA	0/1	128
Case report	1	5	Carbamazepine	2	Petr	Right after	0/1	
	1	5	Carbamazepine	1	Petr	3 mo	1/1	101
Case series	4	12	Carbamazepine	0.1–100	Petr/ace	0, 1, 4, 5 and 6 y	4/4 (100)	129
Case report	1	0	Carbamazepine	0.1–10	Per	4 mo	0/1[d]	130
Case report	1	5	Carbamazepine	1 and 5	Meth	NA	1/1	89

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Table I. Contd

Type of study	No. of pts	No. of controls	Drug[a]	Conc. [w/v] (% unless otherwise indicated)	Vehicle	Time[b]	Frequency of positive result (%)	Reference
Case series	5	20	Carbamazepine	1	Petr	3 mo–5 y	4/5 (80)	84
Case series	3	0	Carbamazepine	10	Petr/eth/DMSO	NA	3/3 (100)	131
Case report	1	9	Carbamazepine	10, 20,40	YSP	3 y	1/1	95
Case series	18	20	Carbamazepine	3, 10	Petr/wat/eth	DUR	9/18 (50)	71
Case report	1	0	Carbamazepine	100	Pure	Right after	0/1	
Case report	1	20	Carbamazepine	0.1, 1	Petr	4 wk	1/1	66
	1	0	Carbamazepine	10	Petr	4 wk	0/1	132
Case report	1	0	Carbamazepine	0.001–5	Petr	NA	1/1[c]	75
Case series	25	10	Carbamazepine, oxcarbazepine	NA	NA	NA	6/25 (24)	51
Case series	6	0	Carbamazepine	0.3–20	Petr/sal	NA	4/6 (67)	
	2	0	Phenytoin	0.3–20	Petr/sal	NA	1/2 (50)	
	10	0	Phenobarbital	0.3–20	Petr/sal	NA	4/10 (40)	
	5	0	Valproic acid	0.3–20	Petr/sal	NA	4/5 (80)	64
Case report	1	0	Carbamazepine	1, 10, 100	Ace/petr	DUR	1/1	90
Case report	1	0	Carbamazepine	Cr.Tab	Petr/wat	DUR	1/1[c]	85
Case report	1	0	Carbamazepine	0.0001–0.1	Petr	6 mo	1/1[c]	76
Case series	10	80	Carbamazepine	1, 5, 10	Petr	NA	3/10 (30)	133
Case report	1	0	Carbamazepine	1–10	Petr	DUR	0/1	100
Case series	3	0	Carbamazepine	1–10	Petr	4–7 mo	3/3 (100)	86
Case report	1	0	Carbamazepine	Pure, 1	Petr/ace	3 mo	1/1	134
Case report	1	0	Phenobarbital	20	Pr. gly	Right after	1/1	91
Case series	7	18	Carbamazepine	10, 20, 40	Petr	14 wk–7 y	6/7 (85.7)	96
Case report	1	4	Phenytoin	1, 5, 10	NA	5 mo	0/1	
	1	4	Carbamazepine	1, 5	NA	5 mo	1/1	135

a The suspected drug causing the reaction as suggested by at least the medical history of the patch test(s).

b Time elapsed between the reaction and the test.

c Positivity depends on concentration and/or vehicle used.

d Patch test with 10% in petr. was slightly positive at 3 days.

Ace = acetone; **Aft. rec.** = after recovery; **Conc.** = concentration; **Cr.Tab** = crushed tablet; **DMSO** = dimethyl sulfoxide; **DUR** = during the reaction; **Eth** = ethanol; **Meth** = methanol; **NA** = not available; **PBS** = phosphate-buffered saline; **Petr** = petrolatum; **Pr. gly** = propylene glycol; **pts** = patients; **Retr.** = retrospective; **Sal** = saline; **Wat** = water; **w/v** = weight by volume; **YSP** = yellow soft paraffin.

the lowest with phenobarbital. These values range from 20% to 80%; however, it is difficult to draw a firm conclusion because in most of the cases it is only the medical history of the patients that provides any evidence of the drug involved. Oral rechallenge would help confirm the identity of the suspect drug, but because of the possible severity of the reaction, a systemic rechallenge is rarely performed.

The PPV of the patch test in the diagnosis of AHS appears to be higher than its NPV. This trend is expected because there are two types of determinants in achieving a positive patch test: (i) the technical and toxicokinetic characteristics of the agent prior to its introduction to the immune cells; and (ii) the readiness of the immune system to recognize this agent and elicit its distinct reaction. Both of these types of factors appear to contribute to the success of the drug in eliciting a positive patch test. In fact, some investigators believe it is quite "astonishing" that the patch test can give a positive reaction at all.[53] This doubt is especially relevant for drugs in which the mechanism of hypersensitivity is believed to involve long and complex pathways. Positive patch test results in AHS can be indicative of patient sensitivity to the drug (high PPV) but negative ones are not conclusive (low NPV) as false-negative results have been described.[136]

The patch test is capable only of detecting a rather strong inflammatory reaction and this capability depends on how many inflammatory components are involved in the hypersensitivity reaction.[53] Therefore, weak or intermediate immune responses are unlikely to be detected by patch testing. Recent advances in genetic research have allowed the discovery of associations between genetic polymorphisms in certain genes (e.g. *HLA-B* and *heat shock protein 70*) and the risk of specific types of drug hypersensitivity reactions.[137-143] However, no genetic marker has yet been identified that has sufficient predictive value to be used as a screening tool for AHS predisposition in the general population.[137] A recent alert has been issued by the US FDA recommending screening all patients with Asian ethnicity for the *HLA-B*1502* allele before prescribing carbamazepine because of the proven genetic association between this allele and a high risk of developing severe forms of hypersensitivity reactions (SJS/TEN).[144] However, the Asian population consists of multiple ethnic groups that vary considerably in terms of genetic composition, including the frequency of the *HLA-B*1502* allele. Furthermore, no link was found between this type of mutation and other non-bullous forms of carbamazepine-induced hypersensitivity reactions, making genetic screening useless in predicting patient susceptibility to these reactions.[145] However, it is of interest that different polymorphic alleles were found to associate with specific forms of hypersensitivity reaction (maculopapular eruption, multiple organ syndrome, SJS, TEN), implying varying pathological mechanisms for each reaction. This may partially explain differences in patch test performance in patients developing different clinical manifestations of AHS.

7. Conclusion

Patch testing is one of the tools that can be used to diagnose or predict AHS. It is apparent that patch testing can detect only a small portion of the immunological reactions that underlie AHS, therefore other diagnostic methods, such as systemic rechallenge, LTA and/or LTT, should be utilized to make testing more reliable. However, the benefit of testing appears to be maximal with certain drugs (i.e. carbamazepine and phenytoin) and for specific clinical manifestations (strong reactions). It should be performed 2–6 months after recovery from the date of the ADR for best results, with adequate vehicle control. In addition, the test procedure must be standardized in order to evaluate its performance in the diagnosis of drug-induced hypersensitivity reactions.

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