

Late reactions in patch tests: a 4-year review from a clinic of occupational dermatology

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Late patch test reactions on day (D) 10–14, which on subsequent retesting appear in the normal time schedule on D2–D4, indicate active sensitization due to the patch test. The objective of this study was to report our series of late patch test reactions. We analysed the clinical records of all the patients who had reported late reactions for the date of the late reaction, the results of the first patch tests and retesting. Between January 2002 and February 2006, we had a total of 16 patients with late reactions ($\geq$ D10). 12 of them were retested. Retesting confirmed active sensitization to 1 or more allergens in 11 patients (1.3% of 884 patients patch tested). In these 11 cases, none of the late reactions had clear relevance to the symptoms before its appearance. The commonest allergens were paraphenylenediamine (PPD) in 6 patients (0.72% of 826 PPD tests), elecampane (*Inula helenium*) and black-eyed Susan (*Rudbeckia* sp. hybr.) extracts in 3 patients, and 4-aminoazobenzene and Disperse Orange 3 in 2 patients. The late reaction did not generally affect the clinical course. Only 1 patient developed new dermatitis possibly due to active sensitization. Late reactions meeting the classic criteria of active sensitization were relatively common in our series from a special clinic of occupational dermatology.

Key words: 4-aminoazobenzene; 4,4'-diaminodiphenylmethane; 4-*tert*-butylphenol formaldehyde resin; active sensitization; Compositae; Disperse Orange; methyldibromo glutaronitrile; *N,N*-diglycidylaniline; octylisothiazolinone; paraphenylenediamine. © Blackwell Munksgaard 2007.

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Active sensitization is a known adverse effect of patch testing. Traditionally, patch test sensitization is detected by a new reaction at the test site 10–14 days after application. On retesting, the reaction appears in the normal time schedule, as early as after 2–4 days (1). However, it has been shown that positive patch tests to gold salts (2), Compositae extracts (3) and methacrylates (4) can appear after 10 days without being due to patch test sensitization. Retesting a patient after an interval may also show patch test sensitization, although the patient has not noticed any late reaction after the first test. In this situation, the patient may also have been sensitized between the two tests due to environmental exposure to the antigen, or the positive reaction on the second test only represents an increased level of sensitivity (5).

We report our series of late reactions from the past 4 years.

Patients and methods

At Finnish Institute of Occupational Health (FIOH), we have a special outpatient clinic of occupational dermatology. Patients come from all parts

of Finland. Most of our patients are sent for re-evaluation to our clinic by an insurance company after they have been patch tested in other clinics.

Patch tests are performed according to the guidelines of International Contact Dermatitis Research Group using the Finn Chamber method. The concentrations, vehicles and suppliers of the test substances with late reactions are given in Table 1. The tests are read on D2–D3–D4, D2–D5 or D2–D3–D6 depending on the day of application. In written instructions, we advise the patients to contact us if they notice late reactions after the last reading. Since 2002, we have recorded all the patients reporting late reactions.

A late reaction was defined as a reaction, which was noticed (by the patient or someone else) at least 10 days after the application of the test, and which was negative in the last test reading on D4–D6 at FIOH. If the patient was able to come to FIOH, we tried to identify the allergen by the anatomical region, skin markings or position to other allergic reactions. If we could not identify the allergens, we performed new patch tests in such an extent that they covered all possible allergens. We analysed the clinical records of the patients with late reactions

Table 1. Test substances which caused late reactions meeting the classic criteria of active sensitization, their concentration, supplier, number of actively sensitized patients, total number of tests and relative number of sensitized patients for individual allergens

Allergen	Concentration per cent in petrolatum	Supplier	Total number of patch tests with the individual allergen	Patch-test confirmed late reactions meeting the classic criteria of active sensitization	
				Absolute number	Percentage from the total number of patch test with the same allergen (%)
Paraphenylenediamine	1	T	826	6	0.72
Elecampane (<i>Inula helenium</i>)	0.1	H	40	3	7.5
Black-eyed Susan (<i>Rudbeckia</i> sp. hybr.)	1	H	40	3	7.5
4-Aminoazobenzene	1	T	85	2	2.4
Disperse Orange 3	1	T	58	2	3.4
Disperse Orange 1	1	C	57	1	1.8
4,4'-Diaminodiphenylmethane	0.5	T	573	1	0.17
Octylisothiazolinone	0.1	C	782	1	0.13
Compositae mix	3	O	867	1	0.12
Sesquiterpene lactone mix	0.1	C	894	1	0.11
4-tert-Butylphenol formaldehyde resin	1	T	827	1	0.12
Methyldibromo glutaronitrile	0.5, 0.3	O, T	741, 825	1, 1	0.13, 0.12
N,N-diglycidylaniline (epoxy compound)	1	O	127	1	0.79

T, Trolab (Hermal, Reinbec, Germany); C, Chemotechnique (Chemotechnique Diagnostics, Vellinge, Sweden); H, provided by Professor Hausen, Buxtehude, Germany; O, own test substance (Compositae mix 3% is diluted from Trolab's Compositae mix 6%, 1,2-dibromo-2,4-dicyanobutane diluted from analytical grade chemical from Schülke et Mayr GmbH; N,N-diglycidylaniline diluted from chemical from Aldrich, CAS no. 2095-06-9).

for the results of the patch tests and the date of the late reactions.

As paraphenylenediamine (PPD) was the commonest allergen causing active sensitization, we also evaluated the importance of testing PPD in our patient material. We searched all our patients with an allergic (+/++/+++) reaction to PPD in our test files and analysed their clinical records for relevance of this allergic reaction, other patch test reactions at FIOH and also the results of patch tests prior to the investigation at FIOH.

Results

Between January 2002 and February 2006 we have patch tested 884 patients. 16 (1.8%) patients reported reactions, which they had noticed on D10 or later. 12 of them were retested (no. 1–12 in Table 2). 1 patient did not want to be retested (no. 13). In 2 cases, we could identify the allergens by the anatomical region, skin markings or position to other reactions with such certainty that we did not consider retesting necessary (no. 14–15). In 1 case with 9 late reactions (no. 16), we gave up retesting because we feared that it could have induced further active sensitization. The occupations, clinical data and the patch test results of the 16 patients are given in Table 2.

Patient no. 12 was probably not actively sensitized: the probable primary allergen, lettuce, was

not tested in the first session; Compositae mix and 4-tert-butylphenol formaldehyde resin gave ?+ reactions on the final reading on D5; Compositae mix, sesquiterpene lactone mix, parthenolide and laurel probably represented cross-allergy to lettuce. Other 11 retested patients met the classic criteria of active sensitization, so 1.3% of our patients were actively sensitized to at least 1 allergen. In Table 1, we present the allergens, their suppliers, the total number of tests for the individual allergens and the number of patch-test confirmed active sensitizations.

When we evaluated the late reactions of the remaining 11 patients, we did not find any clear relevance to the symptoms before their appearance in the first tests at FIOH. One reaction to PPD had possible relevance: patient no. 5 had had 'irritation' from dyeing her eyelashes. None of the patients with late PPD reactions was a hairdresser. The relevance of the late reactions to Compositae extracts, sesquiterpene lactone mix and Compositae mix cannot be excluded, because they occurred in patients who had handled various decorative plants (not elecampane or black-eyed Susan) or lettuce, but we considered it unlikely. We cannot exclude possible relevance of the late reaction to methyldibromo glutaronitrile either (patient no. 10), because it a common antimicrobial agent, but we did not find it in the products the patient had used.

Table 2. Patients who reported late patch test reactions after first tests at FIOH, their occupation, allergic patch test reactions at FIOH, time to preceding patch tests at local hospitals, number of late reactions and the date they were noticed, and the allergens which were identified without retesting

Patient no., occupation	Time from the earlier patch tests at a local hospital	Result of the first patch test at FIOH [D2-(D3)-D4/5/6]	The number and date of the late reactions ($\geq$ D10)	Result of the second patch test at FIOH [D2-(D3)-D4/5/6]
1, carpenter	6 months	—	1 reaction, D18	PPD +
2, farmer	7 months	—	1 reaction, D11	PPD +
3, textile shop assistant	6 years	— (nickel and cobalt NT)	3 reactions, D16	PPD +++, Disperse Orange 3 ++, Disperse Orange 1 +, 4-aminoazobenzene +++ PPD +++, 4-aminoazobenzene ++, Disperse Orange 3 ++
4, shop assistant (second-hand clothes)	ND (5 months to open test with textile colours)	—	3 reactions, D30	PPD +++, 4-aminoazobenzene ++, Disperse Orange 3 ++
5, assistant nurse	7 months	— (hydrocortisone 17-butyrate, nickel and cobalt NT)	3 reactions, D14	PPD ++, 4,4'-diaminodiphenylmethane ++, octylisothiazolinone ++
6, teacher of boat building	ND	Lyril +	2 reactions, D22	PPD +++, <i>N,N</i> -diglycidylaniline +++
7, chef	21 months	Formaldehyde ++ (lettuce –)	2 reactions, D14–16	Elecampane +++, black-eyed Susan +++ (3 new D10 reactions to Compositae plant extracts)
8, gardener	10 months	—	4 reactions, D19	Elecampane +++, black-eyed Susan +++, sesquiterpene lactone mix ++, 4- <i>tert</i> -butylphenol formaldehyde resin +
9, florist	ND	Nickel sulfate +++	2 reactions, D11 and D14	Elecampane +++, black-eyed Susan +++
10, gardener	12 months	Tuliposide A +, tulipaline A ++, diallyldisulfide +, thymoquinone ++	3 reactions, D28	(Standard series and dilution series of methyldibromo glutaronitrile) methyldibromo glutaronitrile 0.5% + and 0.3% +
11, cook/waitress	6 months	— (lettuce NT)	1 reaction, D14	Compositae mix ++
12, cook	7 and 36 months	Compositae mix ?+, PTBPFR ?+, (lettuce NT)	4 reactions, D14	Parthenolide ++, sesquiterpene lactone mix +, laurel ++, (Compositae mix ++, PTBPFR ++, lettuce ++, new test substance)
Late reactions without confirmation by retesting				Identification by position to other allergic reactions
13, dentist	2 and 5 years	Nickel sulfate ++, balsam of Peru +, fragrance mix +, palladium chloride +, gold sodium thiosulfate ++	2 reactions, D13	2 rubber chemicals (possibly cyclohexyl thiophthalimide 2% and 1%)
14, office secretary	ND	PPD +	2 reactions, D15 and D23	Caine mix III (D15 +), TEGDA (D15 –, D28 +)
15, assistant nurse	10 months	Formaldehyde +, CMI +, Quaternium 15 +	3 reactions, D13	PPD, primin, 1 antimicrobial (possibly glyoxal)
16, gardener	50 months	—	9 reactions, D11–D15	

FIOH, Finnish Institute of Occupational Health; PTBPFR, 4-*tert*-butylphenol formaldehyde resin; ND, not done; NT, not tested; PPD, paraphenylenediamine; TEGDA, triethyleneglycoldiacrylate; CMI, chloromethylisothiazolinone.

15 of the 16 patients in Table 2 were followed for some months (range 2–9 months, median 6 months) at FIOH. In the follow-up visit, 7 patients had no dermatitis. 7 patients still had some dermatitis, but the symptoms were similar or milder when compared with those before the appearance of the late reactions. Only 1 patient (no. 3) with late reactions to PPD, Disperse Orange 3, Disperse Orange 1 and 4-aminoazoben-

zene had developed eczema on her groins which was possibly related to her black underwear.

During the same time period, we tested 826 patients with PPD, and 26 (3.1%) of them had a positive reaction (+/++/+++) to PPD in the normal time schedule on D2–D6. In 13 of these 26 patients, the PPD allergy had been diagnosed in earlier patch tests, so we observed 13 new allergic reactions. 9 of these 13 patients had been patch

tested prior to our investigations, and 1 had noticed a late reaction 2 weeks after the patch test at a local hospital. 1 of these 13 patients had had clear skin symptoms from dyeing her eyelashes and 1 patient had had mild symptoms from dyeing her hair. In 1 of these 13 patients, the PPD reaction possibly represented cross-allergy to 4,4'-diaminodiphenylmethane and in 1 patient to black rubber chemicals. In the remaining 8 patients with new PPD reactions, we could not find any relevance in their clinical records. Regarding the 13 previously diagnosed PPD allergies, one hairdresser had occupational allergic contact dermatitis from hair dyes, 1 patient had had symptoms when dyeing her hair, 1 patient had possibly had symptoms from coloured clothes (textile colours were not tested) and 1 possibly from coloured shoes. In 3 of these 13 cases, PPD allergy probably represented cross-allergy to textile colours. 2 patients had dyed their hair or eyelashes, but had not had any symptoms. In 4 cases, there was no mention of PPD exposure or symptoms.

Discussion

Active sensitization is only one possible explanation for a late reaction. There are true late reactions (4, 6–8), and patch test reactivity may fluctuate considerably on subsequent testing due to technical and immunological reasons (9). If the clinical relevance of a late reaction is obvious, pre-existing sensitivity may be a more likely explanation than active sensitization (10).

As the present series is comprised of routinely patch-tested patients, we did not perform readings after D4–D6 unless the patient reported a new reaction. Probably there were late reactions that the patient never noticed. It is difficult to see late reactions on the back, especially if there are several itchy normal allergic reactions. The date of notice of a late reaction is not necessarily the day of its appearance. The late reactions do not always itch, and they might have appeared several days earlier than they were noticed. Particularly those reactions that were noticed soon after D9 might have been late-appearing true-positive reactions and not active sensitizations. However, we included only reactions that were noticed on D10 or later, while in many studies active sensitization has been suspected in reactions appearing much earlier (11, 12). On the whole, we believe that the present series underestimates the true frequency of active sensitization in our clinic.

Identifying the active sensitizer with the second patch test may lead to 'double active sensitization' (13, 14). Patient no. 7 (Table 2) developed 3 new reactions on D10 of the second patch test, but

these new reactions were to *Compositae* extracts, with cross-allergy to *elecampane* and black-eyed Susan, to which she had already been actively sensitized in the first patch test session. We believe that such late reactions to cross-reacting allergens are not true active sensitizations, but (repeated) patch testing possibly induces this widening of reactivity to cross-reacting substances. The same happened to patient no. 12 (Table 1); she developed 5 reactions instead of 4 on the second patch test, and the extra allergen was one of the cross-reacting test substances, laurel, parthenolide or sesquiterpene lactone mix. Her primary allergen was lettuce, which was not tested in the first session. These 2 cases led us to think one possible explanation for late reactions: the primary allergen may remain unknown, and we only observe late reactions to cross-reacting allergens.

Active sensitization has generally been considered to be a rare adverse effect of patch testing (15). In a recent investigation of the German Contact Dermatitis Research Group (GCDRG), PPD elicited late ($\geq$ D7) reactions in 1.5% of routine patch tests (12), and the authors supposed that the majority of these reactions were caused by patch-test sensitization. Our 6 cases of patch-test sensitization to PPD, which were confirmed by retesting, represented 0.72% of all the PPD tests. If we also include 1 D13 reaction, which was not confirmed by retesting, the percentage increases to 0.85%. Our figures are not so far from those of the GCDRG study, considering that we probably have missed some cases when we did not perform any routine readings after D4–6.

PPD belongs to frequent active sensitizers, and its removal from the standard patch test series has been recommended previously (11). In accordance to the members of the GCDRG (12), we have also used Hermal's PPD patch test substance of controlled quality. The patch test concentration of PPD has been 1% for decades (1). It is possible that the actual concentration in the test substance has increased as the raw materials have become purer. The possible differences in the actual PPD concentrations in the patch test substances from different suppliers might explain differences in the figures of active sensitization in different centres (16). The GCDRG has already removed 1% PPD from the German standard series and decided to take efforts to optimize the patch-test conditions of PPD, by reducing either the exposure time to 2 days or the patch-test concentration (12). Our high level of active sensitization to PPD is unacceptable, considering our low number of relevant PPD reactions. Retesting causes extra work, direct and indirect expenses (e.g. sick leave). As reducing the exposure time is not practical,

we favour reducing the concentration. According to our experience with other active sensitizers, reducing the concentration to 50% of the original might not be enough if the aim is to prevent active sensitization. We intend to reduce our PPD concentration to 0.3%.

A Dutch study with PPD and 4-aminoazobenzene in the standard patch test series suggested that combined testing of para-compounds may increase the risk of active sensitization and combined sensitization to different azo dyes (11). Our experience also favours this concept as 3 patients (no. 3–5) developed active sensitization at the same time to PPD and to other cross-reacting para-compounds, 4-aminoazobenzene, Disperse Orange dyes (17) or 4,4'-diaminodiphenylmethane. 4-aminoazobenzene, Disperse Orange 1 and Disperse Orange 3 induced late reactions in much higher percentages of patients than PPD (Table 1). It is possible that these textile colours were primary active sensitizers and concomitant late reactions to PPD only represented cross-allergy.

Sesquiterpene-lactone-containing extracts like *Frullania* or *Compositae* plant extracts are known to induce patch test sensitization (18–21). Extracts of elecampane and black-eyed Susan sensitized 3 of our patients, 7.5% of all patients tested with plant extracts. Consequently, we have already removed these 2 test substances from our plant extract series, as we had already reduced the patch test concentration of elecampane from 1% to 0.1% after a previous case of active sensitization (21). In another report from the year 2001, at FIOH there were 3 active sensitizations to *Compositae* mix and 1 to sesquiterpene lactone mix (19). Both *Compositae* mix and sesquiterpene lactone mix have been in our standard patch test series, but after these previous sensitizations the concentration of the commercial 6% *Compositae* mix test substance was halved to 3% by adding petrolatum to the mix. In the present series, we still had 1 clear case of active sensitization to the diluted 3% test substance (no. 11 in Table 2). Although possibly being more sensitive than sesquiterpene lactone mix, *Compositae* mix has not generally been recommended for screening *Compositae* dermatitis because of its irritant and sensitizing properties (22, 23). Sesquiterpene lactone mix, in turn, is considered a safe and non-irritant screening substance (3), and it is included in the European Standard series. In the present series, we had a gardener who was actively sensitized to sesquiterpene lactone mix, elecampane and black-eyed Susan extracts (no. 8 in Table 2).

4-*tert*-Butylphenol formaldehyde resin has been reported to cause active sensitization (24), but as far as we know there are no previous reports of

active sensitization to octylisothiazolinone or methyltribromo glutaronitrile.

Testing patients' own substances has been considered especially hazardous (15). We test almost all of our patients with products from their workplace (25), but their own products did not induce any active sensitization during the 4-year period. As far as we know, we have not either previously induced active sensitization to patients' own products.

Our patient material is strongly selected for occupational skin disease. Most of the patients in the present series had other contact allergies than those, which were induced by patch testing. Many of our patients are possibly individuals with a general disposition to contact allergies and a higher risk for active sensitization. Most of our patients have been patch tested in other clinics when they come to our evaluation, and the repeated patch tests are another possible explanation for the large number of active sensitizations in our clinic. The time interval between the previous test and the first test at FIOH is usually at least 6 months (Table 2). We keep at least 2-month interval between 2 patch test sessions at FIOH, but retesting from other reasons than late reactions did not induce any active sensitization. We also test a large number of allergens at the same time, which may also increase the risk to active sensitization.

There is a general experience that a contact allergy acquired at patch testing extremely rarely leads to clinical symptoms and does not generally affect the course of the dermatitis (1, 5). A recently reported 14-year series of 7619 patients from Denmark (10) and our experience are also in accordance with this concept. In the present series, only 1 of our patients had developed symptoms possibly due to sensitization to textile colours (patient no. 3).

There are no alternatives for patch tests in the diagnosis of allergic contact dermatitis. Although late reactions occur, possible active sensitization does not usually cause any disease to the patient. When a dermatologist makes a correct diagnosis of allergic contact dermatitis by patch testing, it often leads to complete and permanent relief of symptoms, which is a relatively rare event in other dermatology. Most of our late reactions were caused by only 2 groups of test substances, the para-amino compounds and the sesquiterpene-lactone-containing preparations, which are already known in the previous studies of late reactions (10, 12).

In conclusion, late reactions meeting the classic criteria of active sensitization were relatively frequent in our series affecting 1.3% of our patients tested. PPD was the most frequent sensitizer, followed by two *Compositae* extracts and azo

dyes. We must emphasize that our patient material is strongly selected, most of our patients have been patch tested earlier, and we usually perform wide tests, including many cross-reacting substances in the same session, and therefore our results should not directly be applied to other dermatological patients.

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