

The outcome of an additional patch-test reading on days 6 or 7

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In a retrospective study (period 1997–1998), the usefulness of an additional patch-test reading on day (D) 6 or 7 was investigated. In 62 out of 760 patients (8.2%), 77 late-positive reactions, those manifest after D3, were seen. 4 late reactions of allergens of which a related allergen reacted earlier (at D2 or D3) in the same patient were not incorporated in the study as late-positive. Allergens most involved in producing late-positive reactions were nickel sulfate (20 reactions), neomycin sulfate (7), tixocortol-21-pivalate (5), p.t. butylphenol formaldehyde resin (5) and Cl+Me isothiazolinone (5). Special attention is paid to ?+ reactions at D6 or D7, because these reactions could add extra information to the outcome of patch-test readings, considering that this might be a group of allergens that has been positive between D3 and D6/7 or become positive later on. When this group is included, the total number of late-positive reactions is 105 in 88 out of 760 patients (11.6%). By doing D3 readings in combination with D6 or D7 readings only, one would miss 24 positive reactions. However, we concluded that it was preferable to have a reading on D2 and D3. An extra reading on D6 or D7 is very useful as it gives additional information in 8.2% of patch-tested patients.

Key words: patch tests; late readings; allergic contact dermatitis; nickel; neomycin; tixocortol-21-pivalate; p-tertiary-butylphenol-formaldehyde resin; methylchloroisothiazolinone+methylisothiazolinone. © Munksgaard, 2000.

Accepted for publication 22 December 1999

In most clinics, patch tests are read on day (D) 2 and 3 or 4 (6). It is however a well-recognized phenomenon that some allergens, such as neomycin (1–7) and corticosteroids (8–10), tend to become positive only after a considerable period of time. As a result, some delayed reactions could be missed when one reads patch tests only at the above-mentioned days. There are, however, some doubts about the benefit of these late readings. Macfarlane et al. (1) noted a 7.2% incidence of positive reactions after D4 and Geier et al. (6) concluded in their study about extra patch-test readings on D4, D5 and D6, that it would be useful to perform an additional patch-test reading on D5. On the other hand, Saino et al. (2) found only a 3% increase in positive reactions after D3 and subsequently concluded that patch-test reading after D3 would be too time-consuming to be adopted routinely.

This retrospective study investigates the incidence of positive patch tests at D6 or D7, which had been negative at D2 and D3. Conclusions are drawn regarding the outcome of recalling patients

at D6 or D7 for an extra patch-test reading. In other words: are the otherwise-missed late reactions important enough for patients to have to spend time and trouble in visiting the hospital for an extra patch-test reading on D6 or D7? Attention is also paid to ?+ patch-test reactions at D6 or D7.

Materials and Methods

Selection of patients and allergens

1254 patients were patch tested in 1997 and 1998 at the Contact Dermatitis Clinic of the Free University Academic Hospital in Amsterdam. We evaluated how many patients had a late-positive reaction to patch tests of the European standard series (ESS) (11) and an additional standard serie (ASS; Table 1). Benzocaine in the ESS was replaced by a mix of caines, caine mix III (Chemo-technique®).

Patients who could not attend for patch-test readings on D6 or D7, because of excessive travelling-time or other practical reasons, were excluded,

Table 1. The additional standard series (ASS); allergens are incorporated in petrolatum, unless mentioned otherwise

1. diazolidinylurea (Germall II) 2%
2. phenol formaldehyde resin (PFR-2) 1%
3. coumarin 5%
4. sorbic acid 2%
5. imidazolidinyl urea (Germall 115) 2%
6. adeps lanae c aqua (lanolin) as is
7. budesonide 0.1%
8. tixocortol-21-pivalate 1%
9. tosylamide/formaldehyde resin 10%
10. arnica tincture 3%
11. carba mix 3%
12. ethylenediamine dihydrochloride 1%
13. thimerosal 0.1%
14. Amerchol L-101 50%
15. cocamidopropyl betaine 1% aq.
16. methylidibromo glutaronitrile 0.3%
17. benzoic acid 5%

as well as patients who were not tested with the European and additional standard series. Patients who were tested with only 1 of the standard series (ESS or ASS) instead of both series were also excluded. After these exclusions, 760 patients were left for investigation, 348 in 1997 and 412 in 1998.

Patch-test readings

Patch-test materials were applied on the back of the patient, using Van der Bend square Chambers® (Van der Bend, Brielle) and Fixomull® tape (Beiersdorf) at D0 (Mondays or Tuesdays) and removed at D2. Readings were carried out on D2 (Wednesdays or Thursdays), D3 (Thursdays or Fridays) and D6 or D7 (next Monday), always by the same persons. The readings were carried out according to the recommendations of the International Contact Dermatitis Research Group (ICDRG) (12).

A late-positive reaction was defined as a change from a negative (–) or doubtful (?+) reaction at D3 to a positive (+, ++, +++) reaction at D6 or D7. We also documented the reactions which changed from negative (–) at D3 to doubtful (?+) at D6 or D7 and the ?+ reactions at D3 which remained ?+ at D6 or D7. We shall discuss this separate group as well. Late-positive reactions of patients who already had positive reactions to another related allergen (e.g., fragrance mix, Myroxylon Pereirae, colophonium, arnica tincture, sesquiterpene lactone mix) at D2 or D3, were not marked late-positive in our study if they did not alter the advice that would have been given to the patient based on the results of D2 or D3. The outcome of the D7 readings in these cases is not important to the patient as the final advice is not influenced.

We also investigated the results of patch-test readings when skipping the D2 reading to restrict the number of patch-test readings to 2.

Results

760 patients aged between 5 and 88 years (mean 41 years) were patch tested and evaluated at D2, D3 and D6 or D7: 468 female and 292 male (ratio 1.6:1).

Incidence of late-positive reactions

77 late-positive reactions were found in 62 out of 760 cases (8.2%) (Table 2). 4 late reactions of allergens of which a related allergen reacted earlier (at D2 or D3) in the same patient were not counted as late-positive.

25 allergens were involved, the most common being nickel sulfate (20 reactions in 760 patients: 2.6%). Other late reactions occurred with neomycin sulfate (7; 0.9%), tixocortol-21-pivalate, Cl+Me-isothiazolinone and p.t.butylphenol formaldehyde resin (5 each; 0.7%); potassium dichromate and p. phenylenediamine base both gave 4 (0.5%) late reactions, cobalt chloride and thimerosal 3 each (0.4%), caine mix III, epoxy resin and budesonide 2 each (0.3%). 11 other allergens gave a single late-positive reaction each (0.1%) (Table 3). A total of 13 patients who had a late patch-test reaction had a concomitant late reaction to another allergen: 9 patients had simultaneous late reactions to unrelated allergens (7 patients to 2 different allergens, 2 to 3 different allergens), 4 patients to related allergens (all to 2 different allergens).

Table 3 also shows the frequency of late reactions in relation to the total number of positive reactions for each allergen (the patients who could not be read at D7 are included), the 'relative incidence'. The relative incidence of neomycin sulfate (41.2%) and tixocortol-21-pivalate (45.6%) is al-

Table 2. Late-positive and late-doubtful reactions

A	D3 –, D6/7 +	D3 ?+, D6/7 +	total
A year 1997	38	4	42
A year 1998	25	10	35
total A			77
B	D3 –, D6/7 ?+	D3 ?+, D6/7 ?+	total
B year 1997	5	9	14
B year 1998	6	8	14
total B			28
total A+B			105

A: late positive reactions (+, ++, +++).

B: late doubtful reactions (?+).

Table 3. Allergens involved in late-positive reactions

Allergen	D3 -, D6/7 +	D3 ?+, D6/7 +	total (D3 - or ?+, D6/7 +)	relative incidence (%)
ESS				
nickel sulfate 5%	16	4	20	10.4
neomycin sulfate 20%	7	0	7	41.2
p.t. butylphenol form. resin 1%	5	0	5	19.2
Cl+Me isothiazolinone 0.01% aq.	2	3	5	15.6
potassium dichromate 0.5%	4	0	4	9.5
p. phenylenediamine base 1%	4	0	4	13.8
cobalt chloride 1%	3	0	3	6.4
caine mix III 10%	2	0	2	8.3
epoxy resin 1%	1	1	2	14.3
mercapto mix 1%	2	0	2	11.8
colophonium 20%	1	0	1	2.2
sesquiterpene lactone mix 0.1%	0	1	1	14.3
thiuram mix 1%	1	0	1	4.2
fragrance mix 8%	1	0	1	0.7
wool wax alcohols 30%	1	0	1	3.0
mercaptobenzothiazole 2%	1	0	1	5.0
ASS				
tixocortol-21-pivalate 1%	4	1	5	45.6
thimerosal 0.1%	2	1	3	21.4
phenol form. resin 1%	2	0	2	20.0
budesonide 0.1%	2	0	2	13.3
carba mix 3%	0	1	1	3.6
Amerchol L-101 50%	1	0	1	3.2
diazolidinyl urea 2%	0	1	1	7.7
adepts lanae c aqua as is	1	0	1	12.5
methylidibromo glutaronitrile 0.3%	0	1	1	3.3

Table 4. Allergens with - or ?+ reaction at D3 and ?+ reaction at D6/7

Allergen	D3 -, D6/7 ?+	D3 ?+, D6/7 ?+	total (D3 - or ?+, D6/7 ?+)	relative incidence (%)
ESS				
fragrance mix 8%	1	4	5	3.3
Myroxylon Pereirae 25%	1	1	2	2.7
formaldehyde 1% aq.	0	2	2	8.3
potassium dichromate 0.5%	2	0	2	4.8
Cl+Me isothiazolinone 0.01%	2	0	2	6.3
primin 0.01%	1	0	1	25.0
sesquiterpene lactone mix 0.1%	1	0	1	14.3
thiuram mix 1%	1	0	1	25.0
cobalt chloride 1%	0	1	1	2.1
nickel sulfate 5%	1	0	1	0.5
methylidibromo glutaronitrile 0.3%	0	1	1	4.2
ASS				
benzoic acid 5%	0	2	2	5.1
cocamidopropyl betaine 1% aq.	0	2	2	4.7
carba mix 3%	1	1	2	7.1
arnica tincture 3%	0	2	2	28.6
imidazolidinyl urea 2%	1	0	1	12.5

most 4× as high as the relative incidence of nickel sulfate (10.4%). Other late-positive allergens with a high relative incidence are p.t. butylphenol formaldehyde resin (19.2%), thimerosal (21.4%) and phenol-formaldehyde resin (20.0%).

We also marked the incidence of ?+ reactions at D6 or D7 of allergens which were - or ?+ at D2 or D3 (Tables 2, 4). 26 patients had a ?+ reaction

at D6 or D7, 2 of these patients had such a reaction to 2 different allergens, 1 to related allergens and 1 to unrelated allergens. Thus, the total number of ?+ reactions at D6 or D7 was 28 in 26 out of 760 patients. 16 allergens were involved, the most common being fragrance mix (5; 0.7%); 8, respectively 7 allergens showed 2 (0.3%) and 1 (0.1%) ?+ reaction at D6 or D7.

Table 5. "D2-only-positive" reactions

	I D2 +, D3 -, D6/7 -	II D2 +, D3 ?+, D6/7 ?+/-	III D2 ?+, D3 -, D6/7 -	IV D2 ?+, D3 ?+, D6/7 ?+/-
year 1997	5	3	45	17
year 1998	10	6	42	39
subtotal	15	9	87	56
total	I+II: 24		III+IV: 145	

Table 6. Allergens involved in "D2-only-positive" reactions

Allergen	D2 +, D3 -, D6/7 -	D2 +, D3 ?+, D6/7 ?+/-	total
ESS			
fragrance mix 8%	2	2	4
methyldibromo glutaronitrile 0.1%	3	0	3
Myroxylon Pereirae 25%	1	1	2
PPD-mix 0.6%	2	0	2
potassium dichromate 0.5%	1	0	1
nickel sulfate 5%	1	0	1
quaternium-15 (Dowicil 200) 1%	1	0	1
colophonium 20%	1	0	1
wool wax alcohols 30%	0	1	1
ASS			
cocamidopropyl betaine 1% aq.	0	3	3
ethylenediamine dihydrochloride 1%	1	0	1
carba mix 3%	1	0	1
tixocortol-21-pivalate 1%	1	0	1
benzoic acid 5%	0	1	1
phonole-formaldehyde resin (PFR-2) 1%	0	1	1

Incidence of "D2-only-positive" reactions

24 positive reactions at D2 only (D2 + + +, + +, + and D3, D4 and D6/7 ?+ or -) were found in 24 out of 760 cases (3.2%) (Table 5). Two D2-only-positive reactions of allergens of which a related allergen became positive later on were not counted as D2-only-positive reactions. 15 allergens were involved, the most common being fragrance mix (4 out of 760 cases: 0.5%). Other allergens which were involved are: cocamidopropyl betaine and dibromodicyanobutane (3 each; 0.4%), PPD mix and Myroxylon Pereirae (balsam of Peru) (both 2; 0.3%). 10 other allergens gave a single D2-only-positive reaction (Table 6).

There were 145 doubtful D2 reactions: 87 reactions were ?+ at D2 and - at D3 and D6/7, 56 reactions were ?+ at D2 and ?+ at D3 and/or D6/7. Many different allergens were involved.

Discussion

Is it worth recalling patients at D6 or D7 for an additional patch test reading?

As 77 positive reactions in 62 out of 760 patients (8.2%) would otherwise have been missed, we con-

clude that it is indeed worthwhile to have a D6/D7 reading. It is not very likely that these late reactions represent a delayed irritant reaction. Delayed irritation generally peaks at D2 or D3 and has been noted with only a few substances, although the clinical aspect and course are difficult to distinguish from an allergic reaction (4, 13). Active sensitization, however, is hard to exclude. However, this phenomenon mostly occurs after 10–14 days (1, 14), although positive reactions occurring only 6 or 7 days after application have also been considered to be an indication of active sensitization (1).

Our study did not stress the issue of relevance but we believe, as Geier et al. (6) already mentioned, that it is better to find some positive reactions for which the clinical relevance is not yet revealed than to miss positive reactions which might indicate contact allergens important for the patient.

Allergens involved

The allergens which gave most late-positive reactions were nickel sulfate (20; 2.6%), neomycin sulfate (7; 0.9%), tixocortol-21-pivalate (5; 0.7%), p.t. butylphenol formaldehyde resin (5; 0.7%), Cl+Me

isothiazolinone (5; 0.7%), potassium dichromate (4; 0.5%), para-phenylenediamine base (4; 0.5%), cobalt chloride (3; 0.4%) and thimerosal (3; 0.4%). Of these allergens, we expected neomycin and tixocortol to be possible late-reactors, as has been indicated by others (1–7). Neomycin is known to have a poor skin-penetration capacity and long persistence in the skin, which are ascribed to neomycin's slowness of absorption through the skin and to the possibility of a reservoir of neomycin being retained in the stratum corneum for a long time (7).

We expected tixocortol to react late, because the anti-inflammatory activity of corticosteroids tend to mask the clinical signs of contact dermatitis. This effect disappears in time and just at later readings the test site becomes eczematous (10). Attributions to a late reaction of corticosteroids are also given to the test concentration, choice of vehicle and poor absorption of these allergens (8, 9).

The late-positive reactions to all the other 23 allergens were "unexpected". Especially the high tally of late-positive nickel sulfate reactions is remarkable (although the total number of nickel positives in our clinic in these 2 years was high as well: 16.0%). In the literature, there are reports of negative patch tests to nickel in patients who give a clear history of reactions to metal jewelry, strongly suggesting nickel allergy (15, 16). In 1979, Van Ketel already suggested the importance of the vehicle in cases of metal sensitization (16). Fischer et al. (17, 18) confirmed that the standard vehicle for metal salts, petrolatum, gives a lack of homogeneous distribution of the metal, probably because the metal salts form small particles in petrolatum. The presence of these particles is 1 of the factors that can influence percutaneous penetration (19) and could be the explanation of a late reaction to nickel sulfate and other metal salts. We found a clear history of a reaction to cheap metals in 16 out of 20 patients who had a late reaction to nickel sulfate. As these patch tests became positive only at D6 or D7, they could have been missed in the studies mentioned above (15, 16). Late-positive reactions to nickel, cobalt and/or potassium dichromate are also found in other studies (1, 2, 3, 6).

?+ reactions at D6 or D7

Special attention should be paid to the ?+ reactions at D6 or D7 (28; 3.7%) that had been – or ?+ at D2 and D3. They could add extra information to the outcome of the patch tests, considering that this might be a group of allergens that may have been positive between D3 and D6 or D7: the reaction could be fading by D6 or D7. Another possibility is that allergens give such weak allergic

reactions that they will not become stronger than ?+. A ?+ reaction at D6 or D7 could also become stronger later on.

If we consider the 28 ?+ reactions at D6 or D7 to be fading late-positive reactions (between D3–D6/7), then the final outcome of late-positive reactions will be $77+28=105$ reactions in $62+26=88$ out of 760 patients (11.6%) (Table 2). This consideration seems reasonable, taking into account the recent study of Geier et al. (6). They showed an increase in positive reactions at D4, D5 and D6, compared to D3, of about 15%, the largest increase being at D5 (18.5%), and concluded it would be useful to perform an additional patch test reading after D3 on D5. In contrast to Geier et al. (6), however, in our study, fragrance mix and Myroxylon Pereirae accounted for the most ?+ reactions at D6 or D7, while they found these allergens to react early. We do not have a good explanation for this.

It is advisable to discuss the relevance of these ?+ reactions with the patient and to inform the patient that sometimes reactions become stronger later on. If so, they should be seen again.

"D2-only-positive" reactions

Because of our conclusion that an extra reading on D6 or 7 is useful, one might wonder if it would be possible to restrict the total number of readings to 2, including a D6/7 reading. Therefore we skipped the D2 reading. The total amount of missed positive reactions by skipping the D2 reading was 24. This is a 1/3 of 77, the number of reactions that would have been missed when no D6/7 reading takes place. Thus we prefer to do both readings at D2 and D3 as well as at D6 or D7. When one cannot do 3 readings, because of practical reasons or patient-related problems, then it is preferable to have readings at D3 and D6 or D7 instead of D2 and D3.

Conclusion

The total gain in positive reactions after D3 at D6 or D7 was 77 reactions in 62 out of 760 patients, 8.2%. Nickel sulfate, neomycin sulfate, tixocortol-21-pivalate, Cl+Me isothiazolinone and p.t. butylphenol formaldehyde resin were mostly involved. The total loss of positive reactions when not reading at D2 is 24. An additional patch-test reading on D6 or D7 is useful, although the total amount of positive reactions at D5 might be higher, as Geier et al. (6) showed in their study. They admitted that their procedure would raise practical problems: with a reading at D5, at least 1 patch test reading will be during the weekend(!).

A reading on D6 or D7 can be a reasonable alternative to overcome this practical problem. Another practical point is that, when only 2 readings are possible for one reason or another, it is better to skip the D2 reading than the D6/D7 reading. Before a final decision is made, a study of patch test readings on both D6 and D7 involving more patients should preferably be performed.

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