

The necessity of a test reading after 1 week to detect late positive patch test reactions in patients with oral lichen lesions

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Abstract

Objectives Establishing the clinical relevance of contact allergy to dental materials in patients with oral lichen lesions (OLL) may be difficult, and tests are often read only on day 3 or day 4; also, concentration of the tested allergens may vary. Several studies on dermatitis patients have shown that additional positive patch test reactions can be found after day 4. Therefore, the aim of the present study was to analyse the frequency of late positive reactions to potential allergens in patients with OLL.

Material and methods Eighty-three of 96 consecutive patients with biopsy-verified OLL were patch-tested with a recently developed lichen series. The patches were removed after 48 h and reactions read 3 and 7 days after application.

Results A total of 129 contact allergies were found, and 26 (20.2 %) of the allergic reactions in 23 patients were seen on day 7 only. The 25.2 % increase in positive test reactions with an additional reading on day 7 in addition to day 3 was statistically significant. Metals were the substances with the highest frequency of late positive reactions.

Conclusions Patients with OLL cannot be considered properly investigated with regard to contact allergy, unless the testing has been performed with mandatory readings on day 3 (or day 4) and day 7.

Clinical relevance Late patch test readings are crucial in order to elucidate the role of contact allergy to dental materials in the aetiology of OLL.

Keywords Epicutaneous tests · Late positive test reactions · Dental materials · Oral lichen lesions

Introduction

Establishing the clinical relevance of contact allergy to dental materials in patients with oral lichen lesions (OLL) may be difficult. The dental materials used for oral rehabilitation usually are permanently cemented, and removal, to evaluate clinical relevance of the particular allergens, may be associated with high risks. Therefore, there is a need for well-designed, controlled clinical studies showing the frequency of contact allergy (including late reading) in patients with OLL.

Dental amalgam has been proposed to be one of the major aetiological factors for developing or maintaining OLL [1–3], in one study even in the absence of a contact allergy to mercury [4]. Also, other substances possibly released from dental materials or substances used in the oral cavity have been proposed to contribute to OLL [3, 5–7]. However, studies differ in the choice of test substances and their concentrations as well as the time for test reading. Therefore, results from different studies are difficult to compare.

In general, around 15 % of allergic patch test reactions to substances in a baseline series are only demonstrated on day 7 (D7 reading) compared to a reading on D3 or D4

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Table 1 Test substances included in the newly developed lichen series [21]

Test substance	concentration in % in petrolatum (w/w)	Test preparation from Chemotechnique or prepared at the Department
1 Methyl methacrylate	2	C
2 Triethylene glycol dimethacrylate (TEGDMA)	2	C
3 Urethane dimethacrylate	2	C
4 Ethylene glycol dimethacrylate (EGDMA)	2	C
5 Bisphenol A-glycidyl methacrylate (bis-GMA)	2	C
6 Dimethyl-4-toluidine	5	C
7 2-Hydroxy-4-methoxybenzophenone	10	C
8 1,4-Butanediol dimethacrylate	2	C
9 Bisphenol A dimethacrylate (bis-MA)	2	C
10 Potassium dichromate	0.5	C
11 Mercury	0.5	C
12 Cobalt chloride	1	C
13 2-Hydroxyethyl methacrylate	2	C
14 Benzoyl peroxide	1	C
15 Nickel sulphate	5	C
16 Eugenol (Symrise GmbH & Co. KG, Holzminden, Germany)	2	D ^b
17 Colophony	20	C
18 <i>N</i> -ethyl-4-toluenesulfonamide	20	C
19 Formaldehyde (Acros organics, Geel, Belgium)	1 ^a	D ^b
20 Tolydiethanolamine	2	C
21 Copper sulphate	2	C
22 Methylhydroquinone	1	C
23 Palladium chloride	2	C
24 Aluminium chloride hexahydrate (Sigma-Adrich GmbH, Steinheim, Germany)	2	D ^b
25 Camphorquinone	1	C
26 Dimethylaminoethyl methacrylate	0.2	C
27 1,6-Hexanediol diacrylate	0.1	C
28 Tinuvin P	1	C
29 Tetrahydrofurfuryl methacrylate	2	C
30 Tin	50	C
31 Titanium (AlfaAesar GmbH & Co. KG, Karlsruhe, Germany)	50	D ^b
32 Calcium titanate (Johnson Matthey, Materials Technology UK, Royston, Herts, England)	10	D ^b
33 Silver sulphate (Janssen Chimica, Geel, Belgium)	10	D ^b
34 Ammonium hexachloroplatinate	0.1	C
35 Glutaraldehyde (Glutaral)	0.2	C
36 Titanium nitride (ChemPur Feinchemikalien und Forschungsbedarf GmbH, Karlsruhe, Germany)	5	D ^b
37 Gold sodium thiosulphate	2	C
38 Epoxy resin	1	C
39 Carvone L-form (Acros organics, Geel, Belgium)	5	D ^b
40 Sodium metabisulfite (ICN Biomedicals Inc., Aurora, OH, USA)	2	D ^b
41 <i>Myroxylon Pereirae</i>	25	C
42 Balsam of Canada (Sigma-Adrich GmbH, Steinheim, Germany)	25	D ^b
43 Ethoxylated bisphenol A glycol dimethacrylate (bis-EMA)	2	C
44 Zinc chloride (Sigma-Adrich GmbH, Steinheim, Germany)	1	D ^b
45 Manganese chloride (Sigma-Adrich GmbH, Steinheim, Germany)	2	D ^b
46 2-Tert-buthyl-4-methoxyphenol (3-BHA)(E320)	2	C
47 Propyl gallate	1	C

Table 1 (continued)

Test substance	concentration in % in petrolatum (w/w)	Test preparation from Chemotechnique or prepared at the Department
48 Tert-butylhydroquinone	1	C
49 Anethole	5	C
50 Eucalyptol	5	D
51 Phenyl salicylate	1	C
52 Cinnamic aldehyde (Bedoukian Research, Dansbury, CT, USA)	1	D ^b
53 Menthol (ICN Biomedicals Inc., Aurora, OH, USA)	2	D ^b
54 Thymol	1	D
55 Eusolex 6300	10	C
56 Eusolex 4360/Escalol 567	10	C
57 <i>P</i> -aminobenzoic acid (PABA)	10	C
58 Propolis	10	C
59 Peppermint oil (Lever Oy, Turku, Finland)	2	D ^b
60 Spearmint oil (Lever Oy, Turku, Finland)	2	D ^b
61 Triclosan (IRGASAN DP 300)	2	C
62 Limonene	1	C
63 Methyl cinnamic aldehyde	1	D
64 Dodecyl gallate	0.25	C
65 Octyl gallate	0.25	C
66 Phthalic anhydride–trimellitic anhydride glycol copolymer	1	D

C Chemotechnique, D Department

^a In water weight/volume

^b Substances purchased from other manufacturers than Chemotechnique

only (Magnus Bruze and Marlène Isaksson, personal communication, 2012). These reactions should be referred to as late reactions. Late-appearing patch test reactions or late positive reactions are defined differently by different authors. One author defined late reactions as one that occurred 5 days or more after the patch test was applied [8], and another author defined a late positive reaction as a reaction appearing after D3 [9]. Substances reported to give late positive reactions are the allergens neomycin [9–12], chromium [13], cobalt [12, 13], nickel [9], palladium and corticosteroids [14]. Other examples are metals from the dental series such as gold [11, 15] and mercury [1]. Among plastics, there are several examples. Acrylates tend to give late-appearing patch test reactions, e.g. 2-hydroxyethyl methacrylate (2-HEMA) and ethyleneglycol dimethacrylate (EGDMA) [16] and also isocyanates [17]. The first synthetic plastic, the resol resin PFR-2 based on phenol and formaldehyde, is another example [18]. Allergic reactions to disperse dyes [19] and dodecyl gallate [11] may also appear late.

In a previous controlled study by our research group, OLL patients showed significantly more contact allergies to gold [20], carvone and mercury than control (dermatitis) patients

[21]. On the basis of this latter study, we hereby report an unexpectedly high frequency of allergic patch test reactions seen on D7 only.

Material and methods

Patients

Ninety-six patients referred for suspicion of OLL at two clinics were asked to participate in a controlled study [20]. Inclusion criteria were based on the fulfilment of the revised and modified World Health Organization (WHO) criteria according to van der Meij and van der Waal [22]. Excluded from the study were patients on systemic corticosteroid treatment, those with lesions limited to the gingiva and those aged 20 years or below. Twelve of the original 96 patients chose not to participate in the patch testing, and one patient had a different histological diagnosis than a lichenoid reaction. Eighty-three (86 %) patients with biopsy-verified OLL finally participated in the study. Mean age was 60.0 years (range, 32–83 years), and 65.1 % were females.

Epicutaneous patch tests

OLL patients were tested with a recently developed lichen series, consisting of 66 relevant substances from the dental series and the cheilitis series of the Department of Occupational and Environmental Dermatology in Malmö, in this article named the Department (Table 1). The substances were purchased from Chemotechnique Diagnostics, Vellinge, Sweden, or prepared at the Department (Table 1).

The substances were applied on the upper back on D0 in IQ chambers® (Chemotechnique Diagnostics, Vellinge, Sweden). For the test preparations in petrolatum (pet), 30 mg was applied, and for liquid test preparations, 25 µL was applied using a micropipette. Patch test chambers were secured with Scanpore tape (Norgesplaster A/S, Vennesla, Norway), removed by the patient on D2 and read on D3 and D7 according to the International Contact Dermatitis Research Group (ICDRG) guidelines [23] by a specialised dermatologist.

Statistics

One-sided Fisher's exact test evaluated the difference in additional positive test results between D3 and D7.

Informed consent and ethical approval

All patients signed an informed consent form. The Regional Ethical Review Board, Lund, Sweden, approved the study per the Declaration of Helsinki and the International Conference of Harmonization (ICH) guidelines on Good Clinical Practice.

Results

A total of 129 contact allergies were found in 83 patients with OLL. Twenty-six (20.2 %) of the allergic reactions in 23 patients were seen on D7 only (Table 2). The group of substances with the highest frequency of late reactions was that of metals.

The number of positive patch test reactions on D3 was 103. With an additional reading on D7, 26 additional positive test reactions were found (Table 3). This 25.2 % increase in positive patch test reactions when reading on D7 in addition to D3 was statistically significant (Fisher's exact test, one-sided: $p = 0.0485$). Thirteen of the 26 late positive patch test reactions were doubtful on D3, and 13/26 were negative on D3 (Table 3). Thirteen of the late patch test reactions (13/26) were weak positive reactions (erythema, infiltration and possibly a few papules; +); 12/26 were strong positive reactions (erythema, infiltration, many papules and possibly a few vesicles; ++), and 1/26 was an extremely positive reaction (intense erythema and infiltration with coalescing vesicles; +++).

Patients were, on D3 and D7, asked for possible worsening of oral symptoms during testing. Eight patients reported

Table 2 Test substances in patients with oral lichen lesions with D7 reactions only

Test substance	Number of OLL patients
Ethoxylated bisphenol A glycol dimethacrylate (bis-EMA)	1 ^a
Bisphenol A-glycidyl methacrylate (bis-GMA)	1 ^a
2-Hydroxy-4-methoxybenzophenone	1 ^b
2-Hydroxyethyl methacrylate (2-HEMA)	1
Ethylene glycol dimethacrylate (EGDMA)	1
Mercury	4
Nickel sulphate	2
Cobalt chloride	4 ^b
Gold sodium thiosulphate	4
Colophony	1
Limonene	1
Carvone L-form	1
Tert-butylhydroquinone	1
Dodecyl gallate	1 ^b
Sodium meta-bisulphite	2

OLL oral lichen lesions

^a One patient with two late reactions

^b One patient with three late positive reactions

worsening. Five of these patients reported a slight change; one reported a slight change between D3 and D7, but on the two days of reading, the symptoms were the same. One patient reported a moderate worsening, and one patient reported that her tongue symptoms were reduced but symptoms in her palate were worse. No one with late positive patch test reactions experienced any worsening of their symptoms. Three patients reported a decline of intraoral symptoms, two with no allergies and one with allergy to gold.

No changes on the skin were observed during the testing.

Discussion

Several studies have shown that additional positive patch test reactions can be found when readings are performed after D4, and the importance of late readings have been suggested by several research groups. In 1978, Mitchell [24] found 20 % more positive reactions on D7 than on D2 testing with the "standard screen chemicals". Macfarlane et al. [13] found 7.2 % more positive reactions after D4 and Jonker and Bruynzeel [9] found 8.2 % more positive reactions to allergens in the European Standard Series and an additional series, when an additional reading was performed on D6 or D7. Paired readings on D4 and D7 were more reliable than those on D2 and D4 [13].

Positive patch test reactions appearing on D7 in humans are not considered a manifestation of patch test sensitisation. On

the other hand, late positive reactions on D10 and beyond might indicate active sensitization [25] but not necessarily so [15, 16].

In the present study, mercury, nickel, gold and cobalt were the test substances with the highest frequency of late positive reactions as in accordance with other studies [1, 9, 11–13]. Late positive reactions were also found for 2-HEMA and EGDMA. Isaksson et al. reported on 2-HEMA and EGDMA, and even later readings were suggested for these substances since 16 and 5.3 %, respectively, of the reactions appeared more than 1 week after test application [16]. In the present study, readings after D7 could possibly have identified more allergies, but this was not practically possible.

Interpretation of results from studies on patients with possible adverse reactions to dental materials is difficult not only by the routine of testing but also because of the variety of symptoms and signs described. Adverse reactions in the oral cavity may comprise symptoms exclusively but may also include redness and swelling as well as the most commonly studied oral mucosal conditions in relation to contact allergy, oral lichen planus (OLP) and oral lichenoid contact reactions (OLCR). OLP and OLCR may be indistinguishable clinically

and histopathologically [26], and in the present study, we pooled the patients under the term OLL.

The day of reading patch tests as well as the concentration of allergens used for patch testing differs between studies making comparisons even more complicated. In a retrospective study on 206 patients with a suspicion of contact allergy to dental metals, only 28 patients had positive test reactions to metals used in dentistry. OLP ($n=9$) and stomatitis were the most prevalent oral manifestations in these patients [27]. The readings were performed on D2, D3 and D4. The number of contact allergies was low compared to corresponding reactions in our previous study [21]; the day of test reading and the concentration of applied allergens differed.

In another study, patients with OLL topographically related to dental fillings, 67 % had positive reactions primarily to mercury but also to gold [28]. Readings were performed on D4. Out of 19 patients with OLL adjacent to amalgam fillings, 78.9 % were sensitised to inorganic mercury and 33.3 % (5/15) of the patients with OLL were positive on D10 or D17 only [1]. They were, however, not read on D7.

In a retrospective study of 46 OLP patients, 54 % had at least one positive reaction and 72 % had clinically relevant reactions

Table 3 Test reactivity on D3 and D7 to the 15 substances with positive D7 reactions only

Test substance	Number of patients	Day 3	Day 7
Ethoxylated bisphenol A glycol dimethacrylate (bis-EMA)	1 ^a	0	+
Bisphenol A-glycidyl methacrylate (bis-GMA)	1 ^a	+?	+
2-Hydroxy-4-methoxybenzophenone	1 ^b	+?	+
2-Hydroxyethyl methacrylate (2-HEMA)	1	+?	+
Ethylene glycol dimethacrylate (EGDMA)	1	+?	+
Mercury	4	+?	++
		0	+
		0	+
		0	++
Nickel sulphate	2	0	++
		0	++
Cobalt chloride	4 ^b	+?	+++
		+?	++
		0	++
		+?	+
Gold sodium thiosulphate	4	+?	++
		+?	+
		0	++
		+?	++
Colophony	1	+?	++
Limonene	1	0	+
Carvone L-form	1	+?	+
Tert-butylhydroquinone	1	0	+
Dodecyl gallate	1 ^b	0	++
Sodium meta-bisulphite	2	0	++
		0	+

+? doubtful reaction, + weak positive reaction, ++ strong positive reaction, +++ extremely strong positive reaction

^a One patient with two late positive reactions

^b One patient with three late positive reactions

[5]. The tests were read on D3 and D4 and not according to ICDRG guidelines.

Patients with lichen planus and lichen lesions had a higher frequency of contact allergy to mercury, chromium ($p=0.028$), cobalt, gold ($p=0.041$) and nickel compared to test results in dermatitis patients presented by the National American Contact Dermatitis Research Group (NACDG) [3]. Here, readings were performed 15–30 min after removal of patches, and a second reading was performed between D4 and D7. Of the 55 OLL patients tested with the British Contact Dermatological Society baseline series and to a specialised dental and metal series, 44 % had one or more contact allergies [29]. Readings were performed on D2 and D4 and often on D7. The authors concluded that the contact allergies found were potentially relevant to the causation of the OLL in some of the subjects but not the only mechanism for induction of the condition. Also, in a study of 24 patients with “OLP-like lesions”, 16 (67 %) showed positive reactions to dental materials [30].

To conclude, twenty-six (20.2 %) of the allergic reactions were seen on D7 only, and this 25.2 % increase in positive test reactions was statistically significant. This finding demonstrates the importance of a second reading later than D3/D4 and preferably on D7 in patients with OLL, in order to elucidate the role of the contact allergy to dental materials in the etiology of OLL. With the present knowledge, patients with oral lichen lesions cannot be considered properly investigated with regard to contact allergy unless the testing has been performed with mandatory readings on D3/D4 and D7.

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Conflict of interest All authors have read and approved the manuscript; contributions to the manuscript are as follows:

Camilla Ahlgren, main author and responsible for all administration including ethical application, scientific compilation as well as clinical dental and main editorial work.

Magnus Bruze, medical supervisor, test readings and manuscript editing.

Halvor Möller, medical supervisor, test readings and manuscript editing.

Marléne Isaksson, medical supervisor, test readings and manuscript editing.

Tony Axéll, oral medicine advisor and clinical dental work as well as manuscript editing.

Rolf Liedholm, oral medicine advisor and clinical work as well as manuscript editing.

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