Monitoring contact sensitization to p-phenylenediamine (PPD) by patch testing with PPD 0.3% in petrolatum

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Summary

Background. Being a contact allergen of general relevance, p-phenylenediamine (PPD) is patch tested in the baseline series. However, PPD 1% in petrolatum may actively sensitize. Patch testing with PPD at 0.35% pet. proved to be safe, as far as active sensitization is concerned.

Objectives. To determine whether PPD 0.3% pet. reliably detects PPD sensitization.

Methods. Patch testing with PPD 0.3% pet. and 1% pet. synchronously was performed in consecutive patients in a multicentre study within the Information Network of Departments of Dermatology.

Results. Altogether, 2042 patients were patch tested. PPD 1% pet. yielded 6.0% positive reactions (n = 123), and PPD 0.3% pet. yielded 4.7% (n = 95). The synchronous reproducibility of PPD reactions was similar as known from parallel patch tests with identical PPD concentrations. The diagnostic properties of PPD 0.3% pet. expressed as reaction index and positivity ratio were good. Of the 123 patients reacting to PPD 1% pet., 32 (26%) had no positive reaction to PPD 0.3% pet. In 22 of these 32 patients (69%), no clinical relevance could be found.

Conclusions. As patch testing with PPD 0.3% pet. is reliable according to our results, we recommend replacing PPD 1% pet. in the baseline series with PPD 0.3% pet.

Key words: active sensitization; CAS 106-50-3; monitoring of contact allergy; patch test concentration; patch testing; p-phenylenediamine.
positive not before day 7 or even beyond in 1.5% of routine patch tests, which may be indicative of patch test sensitization (5). Although there is no doubt that PPD fulfills the criteria to be included as an allergen of the baseline series, the DKG removed PPD 1% pet. from the German baseline series in January 2005, because the risk of active sensitization was considered to be too high. As a consequence, contact sensitization to PPD could no longer be monitored as before, because only aimed patch testing in patients highly suspected to be allergic to PPD was performed. In most other countries, PPD continues to be patch tested at 1% pet. in the baseline series, because active sensitization to PPD is not acknowledged as a frequent and clinically relevant problem (6–10).

In order to determine whether patch testing with PPD at lower concentrations would be more suitable, the DKG performed a corresponding study (11). From this study, it was concluded that patch testing with PPD at 0.35% pet. is safe, as far as active sensitization is concerned. Additionally, the results of this study did not point to diagnostic inferiority of this test concentration as compared with PPD 1% pet.

However, as the number of patients involved in this study was limited, the DKG intended to ensure that a PPD test preparation with this low concentration is sufficiently sensitive to monitor PPD allergy epidemiologically, which would be a prerequisite for the reintroduction of PPD to the German baseline series (12). Therefore, we patch tested consecutive patients with PPD 0.3% pet., and performed intraindividual comparisons of patch test results with the established and approved patch test preparation PPD 1% pet.

**Patients, Materials, and Methods**

From November 2008 to December 2009, 15 departments of dermatology, all of them members of the DKG and the Information Network of Departments of Dermatology (IVDK), took part in the study. In consecutive patients, PPD 1% pet. and PPD 0.3% pet. were patch tested synchronously after informed consent had been obtained. Patch tests were performed and read according to DKG guidelines (13). As a carrier system, Finn Chambers® on Scanpor® tape was used in all but one centre, in which Haye’s test chambers were applied in 40 patients, that is, 2% of the total test population. From an unpublished IVDK data analysis, it was known that no systematic error is introduced when data from patch tests carried out with these two different test systems are pooled. Test chambers were filled with a string of the pet.-based test preparation that corresponded to a weight of \( \sim 20 \text{ mg} \), according to published data (14). The patch test exposure time was 48 hr in all patients. PPD 1% pet. was purchased from Almirall Hermal (Reinbek, Germany). PPD 0.3% pet. was prepared by dilution. Samples of PPD 0.3% pet. were analysed at the quality control laboratory at Almirall Hermal in December 2009. PPD contents ranged from 0.297% to 0.325%.

Altogether, 2042 patients were enrolled in the study. A description of the test population according to the MOAHLFA index is given in Table 1. Participating centres and the number of patients in each centre are given in Table 2. It can be seen that there were more patients with occupational dermatitis and hand dermatitis and fewer patients with leg dermatitis among those taking part in this study than in the total IVDK patch test population of 2009. This is attributable to the participation of several centres specializing in occupational dermatology.
Patch test results and clinical data were documented and collected according to the IVDK routine procedure (15). Data were subjected to the IVDK quality control routine (16). For data analysis, patch test reactions at D3 were selected. In a few exceptional cases, where a reading was performed at D4 instead of D3, the D4 reaction was chosen. In most departments of dermatology, no D7 readings are performed routinely. As our focus was to determine whether PPD is suitable for routine screening with the baseline series, we did not perform readings at D7 in this study, because this would not be relevant for most of the patients patch tested later on. Reaction index (RI) and positivity ratio (PR) were calculated, together with their 95% confidence intervals (CIs), according to the original descriptions (17–19). Cohen’s weighted kappa was calculated to estimate the agreement of patch test results obtained with PPD 1% pet. and with PPD 0.3% pet. in intraindividual comparisons. Data analysis was performed with the statistical software package SAS 9.3 (SAS Institute, Cary, NC, USA).

The clinical relevance of positive reactions to PPD was determined by patient interview and/or studying data on the patient’s history on file. A clinically relevant reaction to PPD was assumed if the patient (i) had a dermatitis following hair dyeing, (ii) was a hairdresser with occupational dermatitis, or (iii) other unequivocal exposure to PPD could be proven.

**Results**

Reaction profiles based on patch test readings at D3 (or D4 in exceptional cases), as well as RI and PR calculated on the basis of these data, are shown in Table 3. No late readings were performed. PPD 1% pet. yielded more positive reactions than PPD 0.3% (123 versus 95; 6.0% versus 4.7%). In addition, RI was higher with PPD 1% pet. (0.43 ± 0.135 versus 0.29 ± 0.155); however, the 95% CIs overlapped, so the difference was not significant at a 5% level. PR was similar with both patch test preparations. From the cross-tabulation in Table 4, it can be seen that 32 patients reacted positively to PPD 1% pet., but not to PPD 0.3% pet. This is a proportion of 26% of those 123 patients reacting to PPD 1% pet. Table 5 gives a detailed picture of the reaction scores for both patch test preparations. Agreement for reactions to both test preparations was almost perfect, as indicated by Cohen’s weighted kappa of 0.82 (95% CI 0.77–0.87).

In 72 of 123 patients (59%) with a positive reaction to PPD 1% pet., this reaction was regarded as clinically relevant. Among the 95 positive reactions to PPD 0.3% pet., there were 64 (67%) clinically relevant reactions, which is not statistically significantly more.

### Table 3. Patch test reactions at D3 (or D4 in exceptional cases), and reaction index (RI), with positivity ratio (PR), with their 95% confidence intervals, to p-phenylenediamine (PPD) 1% pet. and PPD 0.3% pet. in 2042 patients synchronously tested

<table>
<thead>
<tr>
<th>Reaction</th>
<th>PPD 1% pet.</th>
<th>PPD 0.3% pet.</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>1870</td>
<td>1895</td>
</tr>
<tr>
<td>?</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>+</td>
<td>64</td>
<td>47</td>
</tr>
<tr>
<td>++</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>+++</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>2042</td>
<td>2042</td>
</tr>
</tbody>
</table>

| RI       | 0.43 ± 0.135| 0.29 ± 0.155|
| PR       | 52% ± 9%    | 49% ± 10%   |

### Table 4. Concomitant reactivity to p-phenylenediamine (PPD) 1% pet. and PPD 0.3% pet

<table>
<thead>
<tr>
<th>Reaction to PPD 0.3% pet.</th>
<th>+, ++, +++</th>
<th>− , ?, IR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction to PPD 1% pet.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+, ++, +++</td>
<td>91</td>
<td>32</td>
<td>123</td>
</tr>
<tr>
<td>− , ?, IR</td>
<td>4</td>
<td>1915</td>
<td>1919</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>1947</td>
<td>2042</td>
</tr>
</tbody>
</table>

IR, irritant reaction. For this table, reactions are dichotomized as positive (+, ++, +++ ) and not positive (−, ?, IR).

### Table 5. Concomitant reactivity to p-phenylenediamine (PPD) 1% pet. and PPD 0.3% pet

<table>
<thead>
<tr>
<th>PPD 0.3% pet.</th>
<th>−</th>
<th>?</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>IR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPD 1% pet.</td>
<td></td>
<td></td>
<td>1861</td>
<td>6</td>
<td>5</td>
<td>15</td>
<td>128</td>
</tr>
<tr>
<td>−</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>IR</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1895</td>
<td>11</td>
<td>47</td>
<td>23</td>
<td>25</td>
<td>41</td>
<td>2042</td>
</tr>
</tbody>
</table>

IR, irritant reaction.

The clinical relevance of reactions in those 32 patients who reacted positively to PPD 1% pet., but not to PPD 0.3% pet., was investigated in detail. Clinical relevance could be established in 10 patients (31%), of whom 4 were hairdressers with occupational hand dermatitis, and 6 were patients with scalp dermatitis after hair dyeing. In 22 patients (69%), no clinical relevance of the reaction to PPD could be found.
Paired patch test reactions in those 10 patients with a clinically relevant positive reaction to PPD 1% pet., but not PPD 0.3% pet., were as follows (PPD 1% pet./PPD 0.3% pet.): +/? in 5 patients, +/irritant reaction (IR) in 2 patients, +/-negative in 1 patient, and +/+negative in 2 patients.

Discussion

According to our results, a patch test preparation with PPD 0.3% pet. is suitable for detecting type IV sensitization to PPD. With an RI of 0.29 and a PR of 49%, it has good parameters of diagnostic selectivity. Statistically, the agreement of test reactions with the well-established and approved test preparation PPD 1% pet. is almost perfect (Cohen’s kappa = 0.82).

There were 26% fewer positive reactions (32/123) than with PPD 1% pet. At first sight, this seems to be a rather high proportion. However, one has to consider that synchronous reproducibility of patch tests is never 100%. The DKG performed a study on this issue in the 1990s (20). Among other allergens, PPD 1% pet. was tested in duplicate synchronously in 1285 patients. Seventy-two patients had at least one positive reaction. Of these, 55 patients (76%) had positive reactions to both patch tests. Seventeen patients (24%) had discordant reactions, namely 16 (22%) positive/negative, and 1 (1%) positive/doubtful. Hence, the proportion of discordant reactions in synchronous patch tests with PPD 1% pet. and PPD 0.3% pet. (26%) is almost exactly the same as in synchronous duplicate patch tests with PPD 1% pet. (24%). However, as can be seen from Table 4, non-reproducibility was not symmetrical in our study. Thirty-two patients reacted to the higher test concentration, but not to the lower one, whereas only 4 patients reacted to the lower concentration but not to the higher one. A reaction pattern like this was to be expected, because strongly sensitized patients would react to both concentrations, whereas – as with other allergens – some of the less intensely sensitized patients would react to the higher test concentration only. However, it is remarkable that the proportion of patients with a clear-cut positive reaction to PPD 1% pet., but not to PPD 0.3% pet., is not higher than the proportion of non-reproducible reactions in synchronous patch testing with PPD 1% pet. (20). The proportion of true cases of PPD allergy identified with PPD 0.3% pet. is the same as with PPD 1% pet. The diagnostic uncertainty arising from non-reproducibility of positive test reactions is the same, whether PPD 1% pet. or PPD 0.3% pet. is used. In addition, in the DKG study presented here, (i) only one-third (10/32) of the reactions missed by PPD 0.3% pet., as compared with PPD 1% pet., were clinically relevant, and (ii) 7 of these 10 ‘missed’ reactions were not fully negative for PPD 0.3% pet., but were doubtful or irritant reactions. This means that weak erythematous reactions (read as ? or IR) to PPD 0.3% pet. may indicate sensitization of low degree, and should not be ignored. Retesting with 0.3% or a higher concentration of PPD (0.5% or 1%) may be helpful. In order to avoid a serious reaction, the patient should be informed that he or she might develop an allergic contact dermatitis after contact with PPD-containing hair dyes. However, if the patient is a consumer with a history of a hair dye reaction or a hairdresser with occupational dermatitis, and the patch test with PPD 0.3% pet. is negative, it is important to patch test with PPD 1% pet. and other relevant hair dye chemicals.

Diagnostic properties of PPD 0.3% pet.

As can be deduced from our study, a patch test preparation with PPD 0.3% pet. does not elicit irritant or doubtful reactions to an unacceptable extent. On the contrary, the RI of 0.29 is good, pointing to good diagnostic properties. The PR of 49% is very good, and does not point to an increased proportion of false-positive reactions. This is supported by the comparison of PPD 0.3% pet. with PPD 1% pet., which showed only four cases of positive reactions to the lower test concentration without positive reactions to the higher one.

False-negative reactions

The issue of false-negative reactions has already been addressed above. To investigate this problem, one has to define an external gold standard, which is almost impossible in the case of PPD allergy, taking into consideration that clinical relevance in terms of identifying the allergen source can be established in only approximately half of the patients sensitized to PPD. Two ways to solve this problem seem to be possible.

First, one could perform investigations in patients with a clear-cut PPD allergy. These are patients suffering from more or less severe dermatitis after contact with hair dyes, be it as customers or hairdressers, or patients with dermatitis following a so-called ‘temporary black henna tattoo’. In both scenarios, it has been shown that patch test concentrations far below 1% PPD, for example 0.1% PPD pet., are sufficient to prove contact allergy in the vast majority of the cases (21–25). From this, one can conclude that PPD 0.3% pet. is also suitable.

Second, one could perform a comparison with the internationally still used, well-established patch test preparation PPD 1% pet. Apart from our study, which proved the good diagnostic properties of patch testing with PPD 0.3% pet., no investigations of this kind have been
conducted up to now. Supporting evidence comes from the above-mentioned Danish patch test study with a dilution series of PPD in pet. (21). In this study, 12 of 15 patients (80%) with known PPD allergy were recognized by patch testing with PPD 0.1% pet., whereas this proportion was 13 of 15 patients (87%) with patch testing with PPD 1% pet. Therefore, this study also emphasizes that, in many cases, patch test concentrations markedly lower than 1% pet. are sufficient to diagnose PPD sensitization.

The DKG study on late patch test reactions with PPD at lower concentrations than 1% pet. (11) showed that the risk of active sensitization decreases with decreasing test concentration. Although there still was a risk (albeit significantly smaller than with PPD 1% pet.) when patch testing was performed with PPD 0.5% pet. or PPD 0.4% pet., no active sensitization was observed with PPD 0.35% pet. There are no other reports of active sensitization caused by patch testing with PPD at low concentrations. Hence, although the database is not very large, one can conclude that the risk of active sensitization with patch testing with PPD at 0.3% pet. is very low, being almost zero.

Conclusion

Patch testing with PPD at 0.3% pet. is safe with regard to irritation, false-positive and false-negative reactions, and active sensitization. We therefore recommend replacing PPD 1% pet. in the patch test baseline series with PPD 0.3% pet. for screening purposes. If the patient has a history that is strongly suspicious for PPD allergy, for instance a hairdresser with occupational dermatitis or a patient with dermatitis after hair dyeing, and the patch test with PPD 0.3% pet. remains negative, then patch testing with a higher concentration is recommended.

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References


22 Malik M, White I R, McFadden J, White J M L. Para-phenylenediamine: testing at 0.01%, 0.1% and 1% in patients with suspected severe hair dye allergy. *Br J Dermatol* 2008: 159 (Suppl. 1): 79.

