Detection of contact allergy: Using more extensive test series increases the diagnostic efficacy of patch tests

Background: Contact allergy is the most frequent type of allergy, affecting 26-40% of all adults and 21-36% of children. The gold standard in the diagnosis of contact allergy is patch test. Objective: To study the influence of the range and composition of patch test series on the efficacy of the diagnostic procedure. Material and methods: Retrospective analysis of the frequency of positive reactions among patients diagnosed with patch tests at our Department during 2 periods: From December 2003 to March 2005, patients were tested with a series of 9 substances plus white petrolatum as the negative control. From April 2005 to July 2008, the series was expanded to 21 substances, while petrolatum was removed. Results: In the analyzed period, 1379 patients were tested with 9 substances plus petrolatum (group referred to as "G9") and 682 patients with 21 substances ("G21"). In G9, at least one positive reaction was observed in 343 (24.9%, 95%CI: 22.6-27.2%) patients, as compared to 376 (55.1%; 95%CI: 51.4-58.7%) in G21 (p<0.001). The increase in the number of tested substances from 9 to 21 led to a significant increase in the mean number of positive reactions per one patient (0.34 in G9 versus 0.90 in G21; p<0.0001). We have not observed any positive reaction to white petrolatum. Conclusions: Patch testing with more extensive test series increases the chance for the detection of patient's sensitizations. As we have not observed any positive reaction to white petrolatum, using the vehicle as negative control does not seem to offer any advantage.

Wstęp: Alergia kontaktowa jest najczęstszym typem alergii, który dotyczy 26-40% dorosłych i 21-36% dzieci. Test płatkowy (patch test) jest złotym standardem w wykrywaniu alergii kontaktowej. Celem pracy była analiza wielkości i składu serii testowej na efektywność diagnostyczną testów płatkowych. Material i metody: Retrospektywna analiza częstości dodatkowych wyników testów płatkowych wśród pacjentów diagnozowanych w Zakładzie Alergologii w Krakowie w 2 okresach: od grudnia 2003 do marca 2005, u pacjentów wykonano testy płatkowe z serii 9 substancji oraz wazeliny biały jako substancją kontrolną. Od kwietnia 2005 do lipca 2008, serię diagnostyczną rozszerzono do 21 substancji, jednocześnie rezygnując z wazeliny jako kontroli. Wyniki: W okresie analizy, u 1379 pacjentów wykonano testy z 9 haptenami oraz wazeliną (grupa określana jako "G9"), a u 682 pacjentów - z 21 substancjami ("G21"). W grupie G9, co najmniej jeden wynik dodatni obserwowano u 343 (24.9%; 95%CI: 22.6-27.2%) pacjentów, a w grupie G21 - 376 (55.1%; 95%CI: 51.4-58.7%) (p<0.001). Zwiększenie liczby testowanych substancji z 9 do 21 zaowocowało znamienitem wzroście częstości dodatnich reakcji, co wskazuje na znaczenie serii testowej na efektywność diagnozowania alergii kontaktowej. Niestety, dodatkowe substancje nie wpłynęły na wyniki testu. Wniosek: Seria testowa zwiększa efektywność diagnozowania alergii kontaktowej, nie wpływa jednak na wyniki testu płatkowego z wazeliną białą.
rally accepted as the method of choice and the "gold standard" in the detection of contact allergy, and in the diagnosis of allergic contact dermatitis [1,13,19]. It helps in identifying and avoiding offending haptens, thus helping in limiting symptoms of the disease [20]. In patients with suspected ACD, PT significantly shortens the time lapse to final diagnosis and increases the chance for full recovery, thus reducing the disease's duration and treatment cost, and positively influencing patients' quality of life [21].

While carrying out PT, the sensitizers (haptens) should be chosen for testing according to clinical history [14]. As not in every case the patient's history is clear enough for the identification of offending sensitizers, "baseline" or "standard" series of haptens are applied in most patients along with suspect substances indicated by clinical picture and history [26]. It may be assumed that composition of patch test series may determine their diagnostic efficacy. In order to verify this, in the present study we have compared the diagnostic efficacy of two routine patch test series of various compositions used in one allergy department.

Methods
We carried out a retrospective analysis of patch test results among all patients diagnosed with PT at the Department of Allergology of the University Hospital in Kra-
kow (Poland) from December 2003 to July 2008. During that period, 2 different series were used as the baseline for patch testing - one consisting of 9 test substances plus white petrolatum (used as negative control), and a second one of 21 test substances (Table 1). Patch substances from Trolab HermaI (Reinbeck, Germany) were applied on patient's dorsum in IQ Chambers (Chemotechnique Diagnostics, Vellinge, Sweden) for 48 h. The read-
nings of test results were carried out after 48 h (Day 3) and 72 h (Day 4) and recorded according to the guide-
lines of the International Contact Dermatitis Research Group (ICDRG) [26].

Table I
Analysis of patch test reactions in compared groups.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Number of patients tested</td>
<td>1379</td>
<td>682</td>
<td>343</td>
<td>376</td>
<td>882</td>
</tr>
<tr>
<td>Number of patients with positive reactions</td>
<td>0.34</td>
<td>p&lt;0.0001</td>
<td>0.90</td>
<td>p&lt;0.0001</td>
<td>0.63</td>
</tr>
<tr>
<td>At least one positive</td>
<td>24.9 (22.6-27.2)%</td>
<td>p&lt;0.0001</td>
<td>55.1 (51.4-58.7)%</td>
<td>p&lt;0.0004</td>
<td>45.5 (41.8-49.2)%</td>
</tr>
<tr>
<td>Nickel sulfate 5% pet.</td>
<td>13.1 (11.3-14.8)%</td>
<td>p&lt;0.0001</td>
<td>27.1 (23.8-30.5)%</td>
<td>--</td>
<td>27.1 (23.8-30.5)%</td>
</tr>
<tr>
<td>Fragrance Mix II 14% pet.</td>
<td>5.9 (4.7-7.2)%</td>
<td>p=0.41</td>
<td>5.0 (3.3-6.6)%</td>
<td>--</td>
<td>5.0 (3.3-6.6)%</td>
</tr>
<tr>
<td>Cobalt chloride 1% pet.</td>
<td>5.4 (4.2-6.6)%</td>
<td>p&lt;0.0001</td>
<td>14.5 (11.9-17.2)%</td>
<td>--</td>
<td>14.5 (11.9-17.2)%</td>
</tr>
<tr>
<td>Colophony 20% pet.</td>
<td>2.3 (1.3-3.1)%</td>
<td>p=0.7</td>
<td>2.1 (1.0-3.1)%</td>
<td>--</td>
<td>2.1 (1.0-3.1)%</td>
</tr>
<tr>
<td>Potassium dichromate 0.5% pet.</td>
<td>2.1 (1.3-2.9)%</td>
<td>p&lt;0.0003</td>
<td>5.1 (3.5-6.8)%</td>
<td>--</td>
<td>5.1 (3.5-6.8)%</td>
</tr>
<tr>
<td>Paraphenylenediamine 0.1% pet.</td>
<td>1.8 (1.1-2.5)%</td>
<td>p=0.003</td>
<td>4.1 (2.6-5.6)%</td>
<td>--</td>
<td>4.1 (2.6-5.6)%</td>
</tr>
<tr>
<td>Thiram Mix 1% pet.</td>
<td>1.2 (0.6-1.8)%</td>
<td>p=0.04</td>
<td>2.5 (1.3-3.7)%</td>
<td>--</td>
<td>2.5 (1.3-3.7)%</td>
</tr>
<tr>
<td>Mercapto Mix 1% pet.</td>
<td>0.9 (0.4-1.5)%</td>
<td>p&lt;0.009</td>
<td>2.3 (1.2-3.5)%</td>
<td>--</td>
<td>2.3 (1.2-3.5)%</td>
</tr>
<tr>
<td>Formaldehyde 1% aq.</td>
<td>1.0 (0.5-1.5)%</td>
<td>p=0.2</td>
<td>0.4 (0.0-0.9)%</td>
<td>--</td>
<td>0.4 (0.0-0.9)%</td>
</tr>
<tr>
<td>White petrolatum</td>
<td>0</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>Balsam of Peru 25% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>Lanolin (Wool alcohol) 30% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>Neomycin sulfate 20% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>PTBF 1% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
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<tr>
<td>Epoxy resin 1% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
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</tr>
<tr>
<td>Benzocaine 5% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>Clorquinol 5% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
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<tr>
<td>Paraben Mix 16% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>MCI/MCI 0.01% aq.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>IPPD 0.1% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
</tr>
<tr>
<td>Mercaptopentobenzoihioole 2% pet.</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Excluded</td>
<td></td>
</tr>
</tbody>
</table>

*95% confidence intervals (95% CI) are given in brackets; *Results of a re-analysis of the G21 group as if they were tested with 9 test substances only (see explanation in the text); "Composition of Fragrance Mix II 14%: α-ethyl cinnamaldehyde 5%, citral 1%, citronellol 0.5%, farnesol 2.5%, coumarin 2.5%, hydroxymethylpentyl cyclohexene carboxaldehyde 2.5%; "Composition of Thiram Mix: tetramethylthiuram disulphide 0.5%, tetraethylhexyl mono-thiophosphate 0.25%, dipentamethylene thiuram disulphide 0.25%; "Composition of Mercapto Mix: dibenzothiazyl disulphide 1%, N-cyclohexylbenzothiazyl sulphenamide 1%, morpholinyl mercaptopentobenzoihioole 0.5%; White petrolatum was used as a negative control; "PTBF, Para tertiary butylphenyl formaldehyde resin; "Composition of Paraben Mix: methyl parahydroxybenzoate 3%, ethyl parahydroxybenzoate 3%, propyl parahydroxybenzoate 3%, butyl parahydroxybenzoate 3% MCI/MCI, 2-methyl-5-chloro-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (3:1 in water); IPPD, N-isopropyl-N-phenyl paraphenylenediamine.

Study group
Altogether, 2061 patients, including 1553 (76%) females and 508 males, aged from 5 to 83 (mean 39) years, were patch tested in the analyzed period. From December 2003 to March 2005, 1379 patients were routinely patch tested with a series consisting of 9 test substances (single haptens or mixtures) and white petrolatum as negative control. This group will be referred to as "G9". From April 2005 to July 2008, 882 patients were tested with a series of 21 test substances ("G21"). The patients were qualified for patch testing by treating doctors as a part of the routine diagnostic procedures, whenever there was a possibility that contact allergy could be a cause of the disease.

Statistical analysis
We have used 2 variables as measures of the efficacy of patch testing in the compared groups: the mean number of positive reactions per one patient, and the percentage of people with positive patch test (detection rate of contact allergy). Doubtful and irritant patch test reactions were excluded from statistical analyses, remaining tests were considered as "positive" regardless of the intensity of reaction (+, ++, or +++ according to ICDRG). Mean numbers of positive reactions per one patient were
References may also be due to random de-
trospective study, because the observed dif-
conclusion cannot be drawn from this re-
situation would be perhaps the most attrac-
chromium, paraphenylenediamine, thiur-
frequency of patients with positive tests, and
ated with the significant increase in both the
hyde resin (PTBF, 2.9%), and epoxy resin
(2.9%), para-tertbutylphenol formalde-
whereas it appeared the third most frequ-
patients with suspected CA. Perhaps most
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substances, as compared to testing with 9
substances. This was observed both when
comparing the real groups (0.34 in G9 vs.
0.90 in G21; p<0.0001), and when re-an-a-
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(0.63 in H9 vs. 0.90 in G21; p<0.0001).
Testing patients from the G21 group only with
9 substances would miss 66 patients (9.7%)
with contact allergy. The positivity rates for
particular haptenst in the compared groups
along with the results of statistical analyses
are shown in Table I. No positive patch test
reactions were observed to petrolatum.

Discussion
The present study demonstrates the impor-
tance of extensive patch testing in patients
with suspected CA. Perhaps most illus-
trative is the case of Peru balsam that
was not included in the "short series" G9,
whereas it appeared the third most frequ-
ent sensitizer in G21 (positivity rate 6.0%).
Further frequent sensitizers, not included in
the G9 series, were: lanolin (4.3%), neomy-
cin (2.9%), para-tertbutylphenol formalde-
hyde resin (PTBF, 2.9%), and epoxy resin
(2.9%). Inclusion of these substances to the
baseline series in the later period contri-
buted to the significant increase in both the
frequency of patients with positive tests,
and the mean number of positive test results per
one patient. In the group G21, there were
higher sensitization rates to nickel, cobalt,
chromium, paraphenylenediamine, thiamur
and mercapto mix. Changing trends in sen-
sitization would be perhaps the most attrac-
tive explanation for this. However, such
 conclusion cannot be drawn from this re-
trospective study, because the observed dif-
ferences may also be due to random de-
mographic differences between patients se-
eking medical help at our Department in the
different time periods, as well as changes in the
medical staff and their diagnostic routi-
nes, and a range other factors. Diepgen and
Coenraads have demonstrated that while te-
sting 2 groups of patients with a test series
of 10 substances, there is a random proba-
bility of over 40% to find, simply by chance,
a statistically significant difference for at le-
ast 1 substance [9]. With respect to the main
goal of the present study, an attempt to over-
come the potential bias connected to the ob-
served differences was undertaken with the
help of the "group H9" model (a re-analysis of
the G21 group, as if they were tested with 9
substances only). This model, which was
free of the random differences between the
real groups, has confirmed the empirical
data.

The present study confirms in a large
group of patients findings from 2 previous
studies, which also demonstrated that te-


testing with more extensive patch test series
leads to detection of more sensitizations,
including those relevant to the patients, and
can improve the efficacy of patch testing in
elicuating causes of contact dermatitis
[7,15]. In diagnostic routines, patch test-
thing should not be limited to standard series
only: In Italy, 41% patients showed positive reac-
tion to test substances not included in the
Italian SIDAPA standard series, consisting
of 21 test substances [11]. In North Ameri-
ca, 15% adults and 39% children showed
positive patch test reactions to substances
not included in the NACDG screening tray
of 50 substances, as well as in T.R.U.E. test
(23 substances) [27]. The British standard
does basically the European baseline
series, supplemented with 12 additional test
substances, each with positivity rates ran-
ning from 0.4-1.6% [2].

Referring to Parent's rule, which states
that in a given relationship 20% of causes are
responsible for 80% of results, an "ide-
al" baseline patch test series should detect
contact sensitizations in at least 80% of pa-
tients. It seems, however, that real life is still
far from this ideal: In 1992, a multi-centre
study revealed that the detection rates for
the contemporary European standard series
ranged from 31-47% [16]. After that study,
aggregation of results of all series included.
The overall positivity rate and the mean
number of positive test reactions was calculated again
for the G21 group, while taking into consideration
only these 9 substances. The results of the described re-as-
semmes are marked further on as "H9", (where "H"
stands for "hypothetical patch test with 9 substances").

Results
Table I shows comparisons between
groups of patients tested with 9 substan-
tes (G9), those tested with 21 substances
(G21), and those tested with 21 sub-
tances, but re-analyzed as if they were te-
sted with 9 substances only (H9). Both the
percentage of patients with positive patch test
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0.90 in G21; p<0.0001), and when re-an-a-
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tive explanation for this. However, such
 conclusion cannot be drawn from this re-
trospective study, because the observed dif-
ferences may also be due to random de-
mographic differences between patients se-
cessity of using the vehicle white petrola-
tum as a negative control in patch tests.
In the initial phase of the analyzed period, white
petrolatum was used along with the routine
series, in analogy to other skin tests, e.g.
prick testing, in which a negative control (ve-
hicle) is routinely used to exclude unspecif-
ically, false-positive reactions. However,
among 1379 patients patch tested with whi-
tet petrolatum, no skin reaction was ob-
v served, suggesting that a possibility of false-
positive reactions to this vehicle virtually
does not exist. False positives in patch test-
ing seem related to the internal character-
istics (irritant potential) of a substance te-
sted, or an increased general irritability of the
skin, rather than to the properties of the
vehicle as such. False positive patch test
reactions due to irritant properties of sub-
stances can be recognized based on the
morphology and time course (e.g. the "de-
crescendo" pattern after removal of the
patch), while false positives due to in-
creased irritancy of the skin should be sus-
p ected when positive reactions to 5 or more
chemically unrelated substances are seen in
a test series [4,17].

Conclusions
Our data demonstrate that patch testing
with more extensive test series improves the
efficacy of the detection of contact sensi-
tizations. From a patient's point of view,
more extensive testing translates into a better
chance of detecting all culprit sensitizers,
thus making the better change for cure. In our
opinion, this benefit fully justifies more exten-
sive patch testing.

Acknowledgements
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