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

Wykrywanie alergii kontaktowej: Rozbudowane serie testowe zwiększają efektywność diagnostyczną testów płatkowych

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Background: Contact allergy is the most frequent type of allergy, affecting 26-40% of all adults and 21-36% 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<0.0001). The increase in the number of tested substances from 9 to 21 led to 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. Materiał i metody: Retrospektywna analiza częstości dodatnich wyników testów płatkowych wśród pacjentów diagnozowanych w Zakładzie Alergologii w Krakowie w 2 okresach: od grudnia 2003 do marca 2005, u pacjentów wykonywano testy płatkowe z serią 9 substancji oraz wazeliną białą 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%) badanych, w porównaniu do 376 (55,1%; 95%CI: 51,4-58,9%) w grupie G21 (p<0,001). Zwiększenie liczby testowanych substancji z 9 do 21 zaowocowało znamiennym statystycznie wzrostem średniej liczby dodatnich odczynów na jednego badanego (0,34 w G9 oraz 0,90 w G21; p<0,001). Nie stwierdzono dodatnich odczynów na wazelinę białą. Wniosek: Im większa liczba substancji w serii testowej, tym większa szansa na wykrycie uczuleń u konkretnego chorego. Ponieważ nie obserwowaliśmy dodatnich reakcji na wazelinę białą, wnioskujemy że stosowanie wazeliny jako kontroli ujemnej nie jest konieczne.

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Background

Contact allergy (CA) is among the most frequent types of allergy, affecting 26-40% adults and 21-36% children [10,22,23,24]. The most frequent clinical manifestation of contact allergy is allergic contact dermatitis (ACD), with the prevalence estimated at 10-17% [18,25] . In our recent study, we have demonstrated that every second child with chronic/recurrent eczema is patch test positive, whereas every third is finally diagnosed with ACD [8]. Patch test (PT) is gene-

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 Krakow (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 Hermal (Reinbek, Germany) were applied on patient's dorsum in IQ Chambers (Chemotechnique Diagnostics, Vellinge, Sweden) for 48 h. The readings of test results were carried out after 48 h (Day 3) and 72 h (Day 4) and recorded according to the guidelines of the International Contact Dermatitis Research Group (ICDRG) [26].

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, 682 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

Table I
Analysis of patch test reactions in compared groups[a].
Analiza wyników testów płatkowych w badanych grupach.

	G9 (2003-2005)	G9 vs G21 - level of significance	G21 (2005-2008)	H9 vs G21 - level of significance	H9 (Hypothetical results in G21) [b]
Number of patients tested	1379		682		682
Number of patients with positive reactions	343		376		310
Mean number of positive test reactions per one patient	0.34	p<0.0001	0.90	p<0.0001	0.63
At least one positive	24.9 (22.6-27.2)%	p<0.0001	55.1 (51.4-58.7)%	p=0.0004	45.5 (41.8-49.2)%
Nickel sulfate 5% pet.	13.1 (11.3-14.8)%	p<0.0001	27.1 (23.8-30.5)%	–	27.1 (23.8-30.5)%
Fragrance Mix II 14% pet. [c]	5.9 (4.7-7.2)%	p=0.41	5.0 (3.3-6.6)%	–	5.0 (3.3-6.6)%
Cobalt chloride 1% pet.	5.4 (4.2-6.6)%	p<0.0001	14.5 (11.9-17.2)%	–	14.5 (11.9-17.2)%
Colophony 20% pet.	2.3 (1.5-3.1)%	p=0.7	2.1 (1.0-3.1)%	–	2.1 (1.0-3.1)%
Potassium dichromate 0.5% pet.	2.1 (1.3-2.9)%	p=0.0003	5.1 (3.5-6.8)%	–	5.1 (3.5-6.8)%
Paraphenylenediamine 0.1% pet.	1.8 (1.1-2.5)%	p=0.003	4.1 (2.6-5.6)%	–	4.1 (2.6-5.6)%
Thiuram Mix 1% pet. [d]	1.2 (0.6-1.8)%	p=0.04	2.5 (1.3-3.7)%	–	2.5 (1.3-3.7)%
Mercapto Mix 1% pet. [e]	0.9 (0.4-1.5)%	p=0.009	2.3 (1.2-3.5)%	–	2.3 (1.2-3.5)%
Formaldehyde 1% aq.	1.0 (0.5-1.5)%	p=0.2	0.4 (0.0-0.9)%	–	0.4 (0.0-0.9)%
White petrolatum [f]	0		Not tested		Not tested
Balsam of Peru 25% pet.	Not tested		6.0 (4.2-7.8)%		Excluded
Lanolin (Wool alcohols) 30% pet.	Not tested		4.3 (2.7-5.8)%		Excluded
Neomycin sulfate 20% pet.	Not tested		2.9 (1.7-4.2)%		Excluded
PTBF 1% pet. [g]	Not tested		2.9 (1.7-4.2)%		Excluded
Epoxy resin 1% pet.	Not tested		2.6 (1.4-3.8)%		Excluded
Benzocaine 5% pet.	Not tested		1.9 (0.9-2.9)%		Excluded
Clioquinol 5% pet.	Not tested		1.3 (0.5-2.2)%		Excluded
Paraben Mix 16% pet. [h]	Not tested		1.2 (0.4-2.0)%		Excluded
MCI/MI 0.01 % aq. [i]	Not tested		1.2 (0.4-2.0)%		Excluded
Quaternium 15 1% pet.	Not tested		0.9 (0.2-1.6)%		Excluded
IPPD 0.1% pet. [j]	Not tested		0.9 (0.2-1.6)%		Excluded
Mercaptobenzothiazole 2% pet.	Not tested		0.6 (0.0-1.2)%		Excluded

[a]95% confidence intervals (95%CI) are given in brackets; [b]Results of a re-analysis of the G21 group as if they were tested with 9 test substances only (see explanation in the text); [c]Composition of Fragrance Mix II 14 %: α-hexyl cinnamaldehyde 5%, citral 1%, citronellol 0.5%, farnesol 2.5%, coumarin 2.5%, hydroxymethylpentyl cyclohexene carboxaldehyde 2.5%; [d]Composition of Thiuram Mix: tetramethylthiuram disulphide 0.25 %, tetraethylthiuram disulphide 0.25 %, tetramethylthiuram monosulphide 0.25%, dipentamethylenethiuram disulphide 0.25%; [e]Composition of Mercapto Mix: dibenzothiazyl disulphide 1%, N-cyclohexylbenzothiazyl sulphenamide 1%, morpholinyl mercaptobenzothiazole 0.5%; [f]White petrolatum was used as a negative control; [g]PTBF, Para tertiary butylphenol formaldehyde resin; [h]Composition of Paraben Mix: methyl parahydroxybenzoate 3%, ethyl parahydroxybenzoate 3%, propyl parahydroxybenzoate 3%, butyl parahydroxybenzoate 3%; [i]MCI/MI, 2-methyl-5-chloro-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (3:1 in water); [j]IPPD, N-Isopropyl-N'-phenyl paraphenylene diamine.

compared in both groups using *t* test. The overall positivity rates (at least one positive patch test reaction in a given patient) and positivity rates for each substance tested were calculated with a 95%-confidence interval (95%CI), and analyzed for possible differences between the groups using Chi^2 test. For statistical tests, $p<0.05$ was selected as the level of significance.

As the initial statistical analysis revealed significant differences between the compared groups G9 and G21 regarding frequency of sensitization to a range of haptens (nickel, chromium, cobalt, paraphenylenediamine, thiuram mix, and mercapto mix), we have realized that these differences might constitute a relevant source of bias for the main outcome of the study. In order to avoid this, we performed an additional analysis, in which we re-analyzed patients from the G21 group, as if they were tested only with the 9 test substances used in G9 group (petrolatum, which produced no positive results, was not 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-assessment 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 substances (G9), those tested with 21 substances (G21), as well as those tested with 21 substances, but re-analyzed as if they were tested with 9 substances only (H9). Both the percentage of patients with positive patch test reactions, and the mean number of positive reactions per one patient was significantly higher while testing patients with 21 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-analyzing the group G21 for 9 substances only (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 haptens 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 importance of extensive patch testing in patients with suspected CA. Perhaps most illustrative is the case of Peru balsam that was not included in the "short series" G9, whereas it appeared the third most frequent sensitizer in G21 (positivity rate 6.0%). Further frequent sensitizers, not included in the G9 series, were: lanolin (4.3%), neomycin (2.9%), para-tertbutylphenol formaldehyde resin (PTBF, 2.9%), and epoxy resin (2.6%). Inclusion of these substances to the baseline series in the later period contributed 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, thiuram and mercapto mix. Changing trends in sensitization would be perhaps the most attractive explanation for this. However, such conclusion cannot be drawn from this retrospective study, because the observed differences may also be due to random demographic differences between patients seeking medical help at our Department in the different time periods, as well as changes in the medical staff and their diagnostic routines, and a range other factors. Diepgen and Coenraads have demonstrated that while testing 2 groups of patients with a test series of 10 substances, there is a random probability of over 40% to find, simply by chance, a statistically significant difference for at least 1 substance [9]. With respect to the main goal of the present study, an attempt to overcome the potential bias connected to the observed 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 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 elucidating causes of contact dermatitis [7,15]. In diagnostic routines, patch testing should not be limited to standard series only: In Italy, 41% patients showed positive reaction to test substances not included in the Italian SIDAPA standard series, consisting of 21 test substances [11]. In North America, 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 series is basically the European baseline series, supplemented with 12 additional test substances, each with positivity rates ranging from 0.4-1.6% [2].

Referring to Pareto rule, which states that in a given relationship 20% of causes are responsible for 80% of results, an "ideal" baseline patch test series should detect contact sensitizations in at least 80% of patients. 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, the series was amended in 1995 (removal of ethylenediamine dihydrochloride, addition of clioquinol and IPPD) [3]. In 2008, European standard series was again expanded through addition of Fragrance mix II (which is different to the "old" Trolab's Fragrance mix II) and Lyral, and renamed to "European baseline series" [5]. The selection of substances for the baseline series is a complicated and continuous process that should reflect changes in epidemiology, appearance of new sensitizers in the environment, and availability of validated test substances [6,12]. At present, the new to European baseline series consists of 28 test substances. Our results confirm that expanding the patch test series seems the right strategy for a cost-effective management of allergic contact dermatitis, especially in the context of the above-mentioned study demonstrating the reduction of disease duration and treatment cost in patch-tested patients with ACD [21].

Our study also hints on the lack of necessity of using the vehicle white petrolatum 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 (vehicle) is routinely used to exclude unspecific, false-positive reactions. However, among 1379 patients patch tested with white petrolatum, no skin reaction was observed, suggesting that a possibility of false-positive reactions to this vehicle virtually does not exist. False positives in patch testing seem related to the internal characteristics (irritant potential) of a substance tested, 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 substances can be recognized based on the morphology and time course (e.g. the "decrescendo" pattern after removal of the patch), while false positives due to increased irritancy of the skin should be suspected 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 sensitizations. From a patient's point of view, more extensive testing translates into a better chance of detecting all culprit sensitizers, thus a better chance for cure. In our opinion, this benefit fully justifies more extensive patch testing.

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