

ASSOCIATED POSITIVE PATCH TEST REACTIONS TO STANDARD CONTACT ALLERGENS: 10-YEAR DATA FROM THE SLOVENIA E-SURVEILLANCE SYSTEM

Authors: Aleksandra Dugonik^{2*†}, Bogdan Dugonik^{1†}, Vili Podgorelec¹, Lucija Brezočnik¹

¹ Faculty of Electrical Engineering and Computer Science, University of Maribor, Koroška cesta 46, 2000 Maribor, Slovenia

² Department of Dermatology, University Medical Centre, Ljubljanska cesta 5, 2000 Maribor, Slovenia

*Correspondence: Aleksandra Dugonik, aleksandra.dugonik@ukc-mb.si, ORCID: 0000-0001-8433-7716

†These authors contributed equally to this work

Abstract:

Background Considerable percentage of patients tested with a standard patch test series respond with a positive reaction to more than one allergen, and some associations between synchronous positive reactions to distinct standard patch allergens have been described in the literature.

Objectives To evaluate the prevalence of sensitization to haptens of the European baseline series as well as the prevalence of oligo- and polysensitization and the most significant associated positive patch test reactions in Slovenia.

Methods Patch testing data collected by the Slovenia E-Surveillance system from January 2008 to December 2017 were retrospectively analyzed.

Results Of a total of 15,171 analyzed patients, 39.29% showed at least one positive reaction. The highest sensitivity rates were noted for metals (Nickel(II)sulfate hexahydrate – 16.33%) and fragrances group (Fragrance mix I – 6.70%). The correlation analysis showed the strongest correlation between Mercapto mix and 2-Mercaptobenzothiazole (MBT) (61.2%), Fragrance mix II and Lyril (50.5%), and Potassium dichromate and Cobalt (II) chloride hexahydrate (33.3%).

Conclusions Sensitization prevalence to the most common haptens was defined, and their strongest correlations on a national level were discovered. Comparison with the European level demonstrates already known associations between allergens according to the model of cross reactivity.

Key words: allergy, contact dermatitis, patch testing, associated reaction, clinical epidemiology, surveillance system, European baseline series

Conflict of Interest: The authors have no conflict of interest to report.

Acknowledgments: The following members contributed data to this analysis. Ljubljana: I. Lunder, T.; Ljubljana II: Kecelj, N.; Maribor: I. Orešič, T.; Maribor: II: Kalač Pandurovič, M.; Celje: Kmecl, T.; Novo mesto: Simončič Godnič M.; Izola: I. Vok, M. The authors acknowledge the financial support from the Slovenian Research Agency (Research Core Funding No. P2-0057).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the [Version of Record](https://doi.org/10.1111/cod.13767). Please cite this article as doi: [10.1111/cod.13767](https://doi.org/10.1111/cod.13767)

Accepted Article

1 Introduction

Contact allergic sensitivity and the consequential allergic contact dermatitis (ACD) still present a serious health problem [1]. Consequently, the European baseline series (EBS) of haptens (contact allergens) was established and is used throughout Europe as an essential patch test screen to diagnose ACD [2]. The primary importance of patch testing is the recognition of individual contact sensitivity. However, evaluating an extensive database of patch testing results enables the identification of epidemiological ACD patterns [3].

Since the population exposure to environmental allergens is continuously changing, the update of the EBS is inevitable. Therefore, the European Environmental Contact Dermatitis Research Group proposed criteria for the inclusion of haptens within the baseline series on behalf of the European Society of Contact Dermatitis (ESCD). Consequently, in the period from 2008 to 2015, 28 of the most relevant haptens were included in the EBS. Afterwards the EBS was extended to 30 allergens with additional haptens Textile dye mix 6.6% pet and Methylisothiazolinone 0.2% aq. [2,4-6].

According to a large epidemiological study, 27% of the representative sample of the general population in five European countries had positive reactions to at least one of the contact allergens included in a series referred to as “baseline series” (previously named “standard series”) [7]. It is also known that a considerable percentage of patients tested with a standard patch test series respond with a positive reaction to more than one allergen, and some associations between synchronous positive reactions to distinct standard patch allergens have been described [8].

Until 2007, the statistical evaluation of patch tests results in Slovenia has been carried out mostly for annual reports and less for the epidemiological survey or additional research work. With the introduction of the national e-surveillance system for contact allergies in Slovenia in 2010 (see Fig. 1), things have pivoted [9].

Patch tests results of patients who had been tested in one of the three main patch tests clinics in the period from 2000 to 2010 were added into the system retrospectively. Multicenter data acquisition on the national level was established within 2010 when the patch tests procedure was unified across all seven skin allergy clinics in Slovenia and the regular use of the national e-surveillance system was introduced. In 2017, the surveillance system was upgraded with the collection of data for the MOAHFLA index. At present (June 2020), the national database contains the data of 25,478 patients.

In this study, we evaluated and described the collected patch testing results of the Slovenia E-Surveillance system covering the period from 2008 to 2017. We also identified significant correlations between positive test reactions in a screening series and compared them to results of other surveillance systems.

2 Materials and Methods

2.1 Database: Slovenia E-Surveillance System

Patch testing results are stored on the Apache Web server (Bloom RB. Apache Server 2.0). Access to the application is secured by encryption and allows login only to the registered users with assigned username and password. The security of the personal data entered into the system is of the highest priority. Thus, the patient's anonymity is guaranteed in compliance with other regulations regarding the protection of personal data according to the requirements of the national information commissioner. Data can be easily transferred to other databases or edited using other software tools. The registered data have been contributed to the European Surveillance System on Contact Allergies (ESSCA) network database since 2012 [9].

2.2 Patch testing method

For this study, we used the patch testing data collected in the period from January 2008 to December 2017. The test chamber of this 10-year period constantly contained 28 haptens of national baseline series as well as EBS (see Table 1).

All 15,171 patients included in the study had been tested with Chemotechnique and AllergEAZE Brial standardized EBS haptens. The test chambers had been attached for 48 hours to do the first reading of results in accordance with standard guidelines, while the second reading had been taken after 96 hours [6]. According to the consensus of clinicians performing patch testing in Slovenia, only the results of the second reading (after 96 hours) are registered in the E-Surveillance System. Only clearly positive reactions (+, ++, +++) are recorded as positive, while irritative or questionable results are recorded as negative.

2.3 Data analysis

Data analysis was performed with the code written in the Python programming language, where two external libraries were used, i.e., SciPy [10] (open-source software for mathematics, science, and engineering) and Pandas [11] (open-source data analysis and manipulation tool). A p -value of less than 0.05 ($p < 0.05$) was considered to indicate statistical significance.

Descriptive statistics was used for the description of the analyzed data, e.g. frequency distribution. To evaluate the strength of the relationship between given allergens, a correlation analysis was conducted. If the correlation analysis shows a high correlation, this indicates a very strong relationship between the two allergens. In the analysis, we estimate the correlation coefficient r that is defined on an interval $[-1, 1]$. The coefficient can be explained as:

$$r = \begin{cases} 1; & \text{higher values of the first allergen are associated with the higher levels of the second one} \\ 0; & \text{absence of correlation} \\ -1; & \text{higher values of the first allergen are associated with the lower levels of the second one} \end{cases}$$

To quantify the strength of the association between two allergens an odds ratio (OR) was calculated, accompanied by the respective 95% ($\alpha=0.05$) confidence interval (CI), as

$$OR = \frac{n_{11}/n_{10}}{n_{01}/n_{00}}$$

where n_{11} is the number of patients with positive reactions to both allergens, n_{10} is the number of patients with positive reaction to the first allergen only, n_{01} is the number of patients with positive reaction to the second allergen only, and n_{00} is the number of patients without positive reaction to either of the two allergens. Standard error is then calculated as

$$SE = \sqrt{\frac{1}{n_{11}} + \frac{1}{n_{10}} + \frac{1}{n_{01}} + \frac{1}{n_{00}}}$$

and the 95% CI as

$$CI_{95} = e^{\frac{\ln \pm Z_{\alpha} \cdot SE}{2}}$$

OR is considered as one of the most common measures for the estimation of individual risk. It addresses the question of how much the frequency or likelihood of a sensitivity (a positive reaction) to an allergen is increased if a patient shows a positive reaction to a certain other allergen, relative to the frequency or likelihood if this positive reaction is not present [12]. Beside the prevalence ratio, OR is the most commonly used measure for estimating individual risk in the field of contact allergy [12]. Fisher's exact test was used to determine if there are non-random associations between the two given allergens.

3 Results

3.1 Demographic data and MOALHFA index

The dataset comprises patch tests results of 15,171 Slovenian patients who underwent patch testing in the period from January 2008 to December 2017. Included in the sample are 4670 (30.78%) males and 10501 (69.22%) females, the average age of patients is 50.34 years.

Since 2017, information regarding MOAHLFA index is also being collected: out of 1705 tested patients, 539 (31.61%) were males, the ratios of the latter were as follows: occupational dermatoses: 41 (3.17%); atopic dermatitis: 124 (9.59%); hand dermatitis: 660 (51.04%); leg dermatitis: 230 (17.79%); face dermatitis: 238 (18.41%), 1059 (81.90%) were aged more than 40.

3.2 Number and proportion of positive reactions

For the analysis of patch tests results, a distribution of the clinical phenotypes based on the number of positive results was regularly conducted. A group of negative patients, at least one positive reaction (P), oligosensitization (proportion of patch tested patient with one or two positive reactions), and polysensitization (proportion of patch tested patients with three or more positive reactions) are well determined [16]. Out of 15,171 cases, 9211 (60.71%) are negative,

5960 (39.29%) are showing at least one positive reaction (known as standardized P-measure), 4891 (32.24%) are oligo-sensitized, and 1069 (7.05%) are poly-sensitized.

The evaluation of patch tests results had also shown only one positive reaction in 3374 (22.44%) patients, 1517 patients (9.99%) had responded with two positive reactions, 650 (4.28 %) with three positive reactions, and the rest (2.58%) had more than three positive reactions. Only 37 (0.24 %) patients had shown more than six positive reactions (see Table 2).

3.3 Sensitivity rates for allergens of EU baseline series

Collected patch test results of 28 different haptens of EBS in the period 2008 to 2017 had shown the sensitivity rates for each allergen listed in Table 1. The highest sensitivity rate is confirmed for Nickel(II)sulfate hexahydrate (16.66%), followed by Fragrance mix I (6.70%), Cobalt (II) chloride hexahydrate (5.89%), Balsam of Peru (5.29%), Potassium dichromate (4.22%), and Fragrance Mix II (3.80%).

3.4 The association between positive reactions to two different allergens: a correlation analysis

In the next step, a correlation analysis was performed to uncover any statistical relationships between two allergens. Fig. 2 represents the correlation analysis of all 28 allergens. The darker the cell is the stronger is the relation. It is evident that some pairs of allergens correlate very strongly. The strongest correlation is between Mercapto mix and 2-Mercaptobenzothiazole (MBT) (61.2%), followed by the correlation between Fragrance Mix II and Lyrall (50.5%), Potassium dichromate and Cobalt (II) chloride hexahydrate (33.3%), Balsam Peru and Fragrance mix I (32.3%), and Fragrance mix I and Fragrance Mix II (31.1%). Table 3 shows the remaining top 15 correlation pairs with the corresponding correlation coefficient.

3.5 The association between positive reaction to two different allergens: odds ratio analysis

Table 4 and Table 5 show the number (n), percentage (%), odds ratios (OR) with 95% confidence interval (95% CI), and the *p*-value (*p*) between two allergens association.

Table 4 delivers associations with a percentage greater than 0.5%, i.e., positive reactions to both allergens noted in more than 0.5% of the patients. The strength of associations between positive reactions to two different allergens was quantified with the OR, accompanied by the respective 95% CI. To account for the effect of multiple testing, the Bonferroni adjustment was used for all statistical tests, namely Fisher's exact test, with adjusted *p*-values of less than .05 considered significant. We can see that there are 24 such associations, and all are statistically significant. The most common two allergens present in these associations are Nickel(II)sulfate hexahydrate and Fragrance mix I, each appearing nine times.

Table 5 shows top 15 pairs of allergens with the highest OR, ten of them having the percentage

rate less than 0.5% of all patients. The highest OR has the association between Mercapto mix and 2-Mercaptobenzothiazole (MBT), for which also the highest correlation has been determined.

4 Discussion

Modern digital electronic data capture (EDC) solutions greatly aid in collecting, combining, and preserving the data in a friendly, undemanding, and time-efficient way for the dermatologists. Creating a central electronic database for patch testing results provides a “single source” documentation strategy and, therefore, a better picture of contact sensitivity and characteristic of allergic contact dermatitis [9]. The Slovenia E-Surveillance system and the German Contact Dermatitis Research Group (Deutsche Kontaktallergie-Gruppe) system of collecting patch-testing data for German-speaking countries represent the only national multicenter surveillance systems of patch testing results in Europe [13].

Comparing the demographic data of the tested population in Slovenia during the period from 2008 to 2017 with ESSCA reports for the period 2007–2008 and 2013–2014 [14,15] shows a slightly lower proportion of males (30.78 %) and patients with age over 40 (69.99%) in Slovenia. A slightly lower proportion (39.9%) of patients with at least one positive reaction, a standardized P-measure, has also been detected. The study of Dittmar et al. [16] shows an equal standardized prevalence of oligosensitization (7.05%) and a slightly lower proportion of the polysensitized population (32.2%) in Slovenia.

In our study, the highest sensitivity rate was confirmed for Nickel(II)sulfate hexahydrate (16.66%), followed by Fragrance mix I (6.70%), Cobalt (II) chloride hexahydrate (5.89%), Balsam of Peru (5.29%) and Potassium dichromate (4.22%), which is comparable with ESSCA results in approximately the same period [16].

Based on the results collected by the ESSCA group for the period 2007–2008 [14], we noted much lower sensitization rates for Nickel(II)sulfate hexahydrate compared to southern EU countries, i.e. Italy and Spain, and the neighboring Austria. Rates of other metals (Potassium dichromate and Cobalt (II) chloride hexahydrate) are comparable to Italian rates; however, they are lower compared to rates in Austria and Spain. Sensitization rates for Fragrance mix I, Fragrance Mix II, and Balsam of Peru are obviously higher compared to Italy; however, much lower compared to rates in Austria. The ESSCA results for period 2013–2014 [15] determine the highest sensitivity for the same haptens (Nickel(II)sulfate hexahydrate 18.1%, Fragrance Mix I 7.3%, Cobalt (II) chloride hexahydrate 5.9%, Balsam of Peru 5.3%, Potassium dichromate 3.2%), but with the obvious discrepancy in Methylisothiazolinone/Methylchloroisothiazolinone (Kathon CG,100 ppm) in ratio ESSCA: Slovenia (7.3%: 3.1%). Compared to the ESSCA results, a higher proportion of Methylidibromoglutaronitrile (MDBGN) sensibilization (3.58%) in Slovenia also stands out [15].

Dickel et al. [17] defined multiple sensitivity as a reaction to more than one allergen and several contact sensitization models. Those are *multiple sensitizations*, where allergens A and B occur in different products and usually induce sensitization; *concomitant sensitization*, where allergens A and B are present in the same product and both induce sensitization; *simultaneous sensitization*,

where allergens A and B are present in different products but may induce simultaneous sensitization; *cross-sensitization*, where A is the primary allergen inducing sensitivity and B is a secondary allergen, chemically related to A and also capable of eliciting contact dermatitis; and *combination of cross-sensitization and concomitant sensitization*.

Concerning the described models [17], our study's most significant associations meet the definition of the combination of cross-sensitization and concomitant sensitization except the association between fragrances and metals, which are, in our opinion, multiple independent sensitizations. When focusing on the most significant associations that have been recognized in statistical evaluations of large patch testing results databases, i.e. the associations between metals of the standard tray [8,17-20], Formaldehyde and its releasers Quaternium 15 [17,21,22], fragrance mix and metals [8,21], Balsam of Peru and fragrance mix [8,21,22], Carba, and Thiurama Mix [19,22], Ethylene Diamine Dihydrochloride and Neomycin [22,23], para-phenyldiamine and Benzocaine [22,23], we can see that our research confirms all of them.

Dickel et al. [17] also reported that despite Nickel sulfate is the most frequent sensitizer, it usually occurs as an isolated allergen with the lowest number of significant positive associations with other allergens. The Mercapto mix, in contrast, has the highest multiple sensitivity index. Our analysis confirms the claim of Nickel sulfate as the most frequent sensitizer, but we cannot confirm it as an isolated allergen, since our results show multiple associations with it. On the other hand, our research shows the same strongest significant associated sensitizations for the pair Mercapto mix – 2-Mercaptobenzothiazole.

Comparing with the results of German Contact Dermatitis Research Group (DKG) [8] with 24 allergens, where 39 combinations of positive reactions occurred in more than 0.5% of patients, we noticed the same most frequent associations between Fragrance Mix I and Balsam of Peru, as well as Cobalt (II) chloride hexahydrate and Nickel(II)sulfate hexahydrate. Our analysis also shows similar strongest significant associated sensitizations as presented in [21,22], i.e. Mercapto mix and 2-Mercaptobenzothiazole, Fragrance mix II and Lyrall, Formaldehyde and Quaternium 15, as well as Benzocaine and 4-Phenylenediamine base.

Top allergen pairs defined by the highest odds ratios for the European region provided by Dittmar et al. [16] match our results. The strongest significant associations in North/Central Europe were found between Quaternium-15 and Formaldehyde, which was also identified with similar odds ratio in our research. In the same North/Central European region, allergen pairs Potassium dichromate – Cobalt (II) chloride hexahydrate and Fragrance mix I – Fragrance mix II were found, which also matches the results of our research. Similarities with Southern Europe were found in the allergen pairs 2-Methoxy-6-n-pentyl-4-benzoquinone (Primin) – Clioquinol (Chinoform, Vioform) and Tixocortol-21-pivalate – Clioquinol (Chinoform, Vioform).

Other strongest associations of our study, i.e. Fragrance mix II – Lyrall, Formaldehyde – Quaternium 15, Benzocaine – 4-Phenylenediamine base, Cobalt (II) chloride hexahydrate – Nickel(II)sulfate hexahydrate, and Fragrance mix I – Fragrance mix II were also confirmed by the studies [8,22,24].

The strongest significant associations of our study are logical and indicate well the connections between haptens which are tested independently in EBS and as an integral part of a mix of haptens, i.e., Mercapto mix and 2-Mercaptobenzothiazole or Fragrance mix II and Lyrar. Linking between Formaldehyde and Quaternium 15, Benzocaine and 4-Phenylenediamine base suggest cross-reactivity that is characteristic for haptens that are transformed in the skin into a chemically similar antigen, i.e. benzocaine, parabens, PPD and azo dyes with a common presence of an amino substituent at the para position of the benzene ring. This structural similarity could explain the frequency of the cross-reaction observed in our patients between PPD and Benzocaine [23] and Formaldehyde and Quaternium 15.

5 Conclusion

This paper presents the evaluation of patch testing results collected in the 10-year period from January 2008 to December 2017 in the Slovenia E-Surveillance System. We identified not only the highest sensitivity rates among tested allergens, but also significant correlations between positive test reactions in a screening series and provided an extended discussion.

Based on the encouraging results and additionally collected data, i.e. anatomical site of dermatitis and occupation from 2017, we are planning to expand the data analysis. The specific goal of our future research will be to further analyze polysensitization. We will do our best to evaluate the prevalence and identify the predictive factors with the use of intelligent data analysis methods. Besides, it would be very informative to conduct an in-depth analysis of polysensitization obtained in our dataset with results of surveillance systems of other counties.

References

- [1] Thyssen JP, Linneberg A, Menné T, Johansen JD. The epidemiology of contact allergy in the general population – prevalence and main findings. *Contact Dermatitis*. 2007;57(5):287-299. doi:10.1111/j.1600-0536.2007.01220.x
- [2] Wilkinson M, Gonçalo M, Aerts O, et al. The European baseline series and recommended additions: 2019. *Contact Dermatitis*. 2019;80(1):1-4. doi:10.1111/cod.13155
- [3] Uter W. Epidemiology of Contact Allergy in Europe: Current Situation and Outlook for the Future. *Actas Dermo-Sifiligráficas (English Edition)*. 2011;102(1):4-7. Doi: 10.1016/S1578-2190(11)70746-6
- [4] Bruze M, Condé-Salazar L, Goossens A, Kanerva L, White IR. Thoughts on sensitizers in a standard patch test series. The European Society of Contact Dermatitis. *Contact Dermatitis*. 1999;41(5):241-250. doi:10.1111/j.1600-0536.1999.tb06154.x
- [5] Isaksson M, Ryberg K, Goossens A, Bruze M. Recommendation to include a textile dye mix in the European baseline series. *Contact Dermatitis*. 2015;73(1):15-20. doi:10.1111/cod.12400
- [6] Johansen JD, Aalto-Korte K, Agner T, et al. European Society of Contact Dermatitis guideline for diagnostic patch testing – recommendations on best practice. *Contact Dermatitis*. 2015;73:195-221. doi:10.1111/cod.12432
- [7] Diepgen TL, Ofenloch RF, Bruze M, et al. Prevalence of contact allergy in the general population in different European regions. *Br. J. Dermatol*. 2016;174:319–329. doi: 10.1111/bjd.14167
- [8] Brasch J, Uter W, Geier J, Schnuch A; German Contact Dermatitis Research Group and the Information Network of Departments of Dermatology in Germany. Associated positive patch test reactions to standard contact allergens. *Am J Contact Dermat*. 2001;12(4):197-202. doi:10.1053/ajcd.2001.26669
- [9] Dugonik A, Kalač Pandurovič M, Vok M, Dugonik B. Development and Significance of the E-surveillance System for Contact Allergies in Slovenia. *Acta Dermatovenereol Croat*. 2017;25(2):91-98.
- [10] Virtanen P, Gommers R, Oliphant ET, et al. SciPy 1.0: Fundamental Algorithms for Scientific Computing in Python. *Nature Methods*. 2020;17:261-272. doi: 10.1038/s41592-019-0686-2
- [11] McKinney W. Data Structures for Statistical Computing in Python. *Proceedings of the 9th Python in Science Conference*. 2010;56-61. doi: 10.25080/Majora-92bf1922-00a
- [12] Uter W, Schnuch A, Gefeller O. Guidelines for the descriptive presentation and statistical analysis of contact allergy data. *Contact Dermatitis*. 2004;51:47-56. doi:10.1111/j.0105-1873.2004.00406.x
- [13] Uter W, Schnuch A, Wilkinson M, Dugonik A, Dugonik B, Ganslandt T. Registries in Clinical Epidemiology: The European Surveillance System on Contact Allergies (ESSCA). *Methods Inf Med*. 2016;55(2):193-199. doi:10.3414/ME15-01-0099
- [14] Uter W, Aberer W, Armario-Hita JC, et al. Current patch test results with the European baseline series and extensions to it from the 'European Surveillance System on Contact Allergy' network, 2007-2008. *Contact Dermatitis*. 2012;67(1):9-19. <https://doi.org/10.1111/j.1600-0536.2012.02070.x>
- [15] Uter W, Amario-Hita JC, Balato A, et al. European Surveillance System on Contact Allergies (ESSCA): results with the European baseline series, 2013/14. *J Eur Acad Dermatol Venereol*. 2017;31(9):1516-1525. doi:10.1111/jdv.14423

- [16] Dittmar D, Uter W, Bauer A, et al. European Surveillance System on Contact Allergies (ESSCA): polysensitization, 2009-2014. *Contact Dermatitis*. 2018;78(6):373-385. doi:10.1111/cod.12966
- [17] Dickel H, Taylor JS, Bickers DR, Merk HF, Bruckner TM. Multiple patch-test reactions: a pilot evaluation of a combination approach to visualize patterns of multiple sensitivity in patch-test databases and a proposal for a multiple sensitivity index [published correction appears in *Am J Contact Dermat*. 2003 Dec;14(4):224]. *Am J Contact Dermat*. 2003;14(3):148-153. doi:10.2310/6620.2003.6166
- [18] Schnuch A, Brasch J, Uter W. Polysensitization and increased susceptibility in contact allergy: a review. *Allergy*. 2008;63(2):156-167. doi:10.1111/j.1398-9995.2007.01590.x
- [19] Albert MR, Chang Y, González E. Concomitant positive reactions to allergens in a patch testing standard series from 1988-1997. *Am J Contact Dermat*. 1999;10(4):219-223. doi:10.1053/AJCD01000219
- [20] Hegewald J, Uter W, Pfahlberg A, Geier J, Schnuch A; IVDK. A multifactorial analysis of concurrent patch-test reactions to nickel, cobalt, and chromate. *Allergy*. 2005;60(3):372-378. doi:10.1111/j.1398-9995.2005.00693.x
- [21] Landeck L, González E, Baden L, Neumann K, Schalock P. Positive concomitant test reactions to allergens in the standard patch test series. *Int J Dermatol*. 2010;49(5):517-519. doi:10.1111/j.1365-4632.2010.04309.x
- [22] Holness DL, Nethercott JR, Adams RM, et al. Concomitant positive patch test results with standard screening tray in North America 1985-1989. *Contact Dermatitis*. 1995;32(5):289-292. doi:10.1111/j.1600-0536.1995.tb00783.x
- [23] González-Rodríguez AJ, Gutiérrez-Paredes EM, Revert Fernández Á, Jordá-Cuevas E. Allergic contact dermatitis to benzocaine: the importance of concomitant positive patch test results. *Actas Dermosifiliogr*. 2013;104(2):156-158. doi:10.1016/j.ad.2011.07.023
- [24] Adler W, Gefeller O, Uter W. Positive reactions to pairs of allergens associated with polysensitization: analysis of IVDK data with machine-learning techniques. *Contact Dermatitis*. 2017;76(4):247-251. doi:10.1111/cod.12706

Table 1: Number of positive reactions and absolute frequencies calculated for each allergen in European baseline series in the period from 2008 to 2017.

#	Allergen	Concentration (%)	Acronym	Number of positive reactions	Absolute frequency (%)
1	Nickel(II)sulfate hexahydrate	0.5 pet	NI	2528	16.66
2	Fragrance mix I	1.0 pet	FMI	1016	6.70
3	Cobalt (II) chloride hexahydrate	1.0 pet	CO	894	5.89
4	Balsam of Peru	20.0 pet	PER	803	5.29
5	Potassium dichromate	1.0 pet	CR	640	4.22
6	Fragrance Mix II	5.0 pet	FII	577	3.80
7	Methyldibromoglutaronitrile (MDBGN)	5.0 pet	DMD	544	3.59
8	Methylisothiazolinone/Methylchloroisothiazolinone (Kathon CG,100 ppm)	5.0 pet	IZO	476	3.14
9	Colophonium	20.0 pet	KOL	450	2.97
10	4-Phenylenediamine base (PPD)	16.0 pet	PPD	413	2.72
11	Hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall®)	0.1 pet	LYR	247	1.63
12	Thiuram mix	30.0 pet	TIU	226	1.49
13	Neomycin sulfate	2.0 pet	NEO	214	1.41
14	Epoxy resin	1.0 pet	EPO	182	1.20
15	Wool (Lanolin) alcohol	25.0 pet	LAN	154	1.02
16	Formaldehyde	1.0 pet	FOR	141	0.93
17	Paraben Mix	2.0 pet	PAR	114	0.75
18	Benzocaine	1.0 aq	BEN	89	0.59
19	Quaternium 15 (Dowicil 200)	8.0 pet	Q15	81	0.53
20	Budesonide	0.1 pet	BUD	81	0.53
21	N-Isopropyl-N-phenyl--4-phenylenediamine	1.0 pet	IFP	81	0.53
22	Sesquiterpene lactone mix	0.01 pet	SQL	80	0.53
23	4-tert-Butylphenolformaldehyde resin (IPPD)	0.01 pet	PBF	74	0.49
24	2-Mercaptobenzothiazole	0.01 pet	MBT	70	0.46
25	Mercapto mix	0.1 pet	MER	67	0.44
26	2-Methoxy-6-n-pentyl-4-benzoquinone (Primin)	0.5 pet	PRI	55	0.36
27	Clioquinol (Chinoform, Vioform)	14.0 pet	KLI	30	0.20
28	Tixocortol-21-pivalate	5.0 pet	TXP	20	0.13

Table 2: Number and proportion of the patients regarding the number of positive patch tests results.

Num. of allergies	Num. of patients	Share [%]
1	3374	22.24
2	1517	10.00
3	650	4.28
4	240	1.58
5	101	0.67
6	41	0.27
7	26	0.17
8	5	0.03
9	4	0.03
10	2	0.01

Table 3: The strongest correlations between allergens.

Allergen 1	Allergen 2	Correlation	p
Mercapto mix	2-Mercaptobenzothiazole (MBT)	0.61	<0.001
Fragrance Mix II	Hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall®)	0.51	<0.001
Potassium dichromate	Cobalt (II) chloride hexahydrate	0.33	<0.001
Balsam of Peru	Fragrance mix I	0.32	<0.001
Fragrance mix I	Fragrance Mix II	0.31	<0.001
Cobalt (II) chloride hexahydrate	Nickel(II)sulfate hexahydrate	0.28	<0.001
Fragrance mix I	Hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall®)	0.23	<0.001
Formaldehyde	Quaternium 15 (Dowicil 200)	0.22	<0.001
4-Phenylenediamine base (PPD)	Benzocaine	0.20	<0.001
Colophonium	Balsam of Peru	0.18	<0.001
Balsam of Peru	Fragrance Mix II	0.15	<0.001
Colophonium	Fragrance mix I	0.13	<0.001
Cl+Me-isothiazolinone (Kathon CG,100 ppm)	Methyldibromoglutaronitrile (MDBGN)	0.12	<0.001
Budesonide	Tixocortol-21-pivalate	0.12	<0.001
Potassium dichromate	Nickel(II)sulfate hexahydrate	0.12	<0.001

Table 4: Analysis of associations. The * symbol indicates statistical significance.

combination	n	%	OR	95% CI	p
CO + NI *	525	3.46	8.72	7.57 – 10.04	<0.001
PER + FMI *	328	2.16	13.73	11.70 – 16.12	<0.001
CR + CO *	277	1.83	17.21	14.43 – 20.52	<0.001
FMI + FII *	264	1.74	15.53	12.98 – 18.58	<0.001
CR + NI *	241	1.59	3.23	2.74 – 3.82	<0.001
NI + FMI *	208	1.37	1.31	1.12 – 1.54	0.001
FII + LYR *	195	1.29	142.76	103.42 – 197.05	<0.001
NI + PER	147	0.97	1.13	0.94 – 1.36	0.206
KOL + PER *	128	0.84	8.27	6.65 – 10.30	<0.001
FMI + LYR *	127	0.84	16.71	12.90 – 21.64	<0.001
PER + FII *	127	0.84	5.81	4.70 – 7.180	<0.001
NI + FII *	125	0.82	1.40	1.15 – 1.72	0.001
KOL + FMI *	115	0.76	5.27	4.22 – 6.58	<0.001
NI + KOL *	110	0.73	1.65	1.32 – 2.05	<0.001
NI + DMD *	110	0.73	1.28	1.03 – 1.59	0.026
NI + IZO *	109	0.72	1.51	1.21 – 1.88	<0.001
PPD + NI *	109	0.72	1.83	1.46 – 2.29	<0.001
FMI + DMD *	108	0.71	3.74	3.00 – 4.67	<0.001
PER + DMD *	100	0.66	4.46	3.55 – 5.61	<0.001
FMI + IZO *	97	0.64	3.84	3.04 – 4.84	<0.001
CO + FMI *	87	0.57	1.55	1.23 – 1.95	<0.001
CR + FMI *	78	0.51	2.01	1.57 – 2.57	<0.001
IZO + DMD *	78	0.51	5.98	4.62 – 7.76	<0.001
CO + PER *	76	0.50	1.73	1.35 – 2.22	<0.001

Table 5: Analysis of the strongest significant associations. The * symbol indicates statistical significance.

combination	n	%	OR	95% CI	p
MER + MBT *	42	0.28	904.56	487.32 – 1679.03	<0.001
FII + LYR *	195	1.29	142.76	103.42 – 197.05	<0.001
BUD + TXP *	5	0.03	66.12	23.44 – 186.49	<0.001
FOR + Q15 *	24	0.16	53.88	32.34 – 89.77	<0.001
PPD + BEN *	40	0.26	32.19	20.94 – 49.49	<0.001
KLI + TXP *	0	0.00	25.20	3.28 – 193.83	0.042
KLI + PRI *	2	0.01	20.33	4.72 – 87.53	0.005
CR + CO *	277	1.83	17.21	14.43 – 20.52	<0.001
FMI + LYR *	127	0.84	16.71	12.90 – 21.64	<0.001
FMI + FII *	264	1.74	15.53	12.98 – 18.58	<0.001
EPO + TXP *	3	0.02	14.76	4.29 – 50.81	0.002
PRI + TXP	1	0.01	14.71	1.94 – 111.88	0.070
PER + FMI *	328	2.16	13.73	11.70 – 16.12	<0.001
PPD + IFP *	21	0.14	13.12	7.90 – 21.79	<0.001
KLI + KOL *	8	0.05	12.09	5.35 – 27.31	<0.001

Figure legends

Fig. 1: Web interface for data entry of tested individuals and marks of positive allergens.

Fig. 2: Correlation analysis.

Patient entry

Name Surname:

Sex:

☒ Male ☐ Female

Year of birth:

1990

Year of testing:

2020

Place of testing:

☒ MB1 ☐ MB2 ☐ CE ☐ IZ ☐ NM ☐ LJ1 ☐ LJ2 ☐ LJ3

Occupation:

☐ KME	☐ PRE	☐ GOS	☐ USN	☐ GUM	☐ PLA	☐ ČIS	☐ LES	☐ GRA	☐ KOV
☐ KOV	☐ FRI	☐ KOZ	☐ ZDR	☒ ZOB	☐ TEK	☐ USL	☐ OST	☐ UPO	☐ NI

Allergens:

☒ CR	☐ BEN	☐ IFP	☐ PBF	☐ Q15	☐ DMD
☐ PPD	☒ NI	☐ LAN	☐ MBT	☐ PRI	☐ FII
☐ TIU	☐ KLI	☐ MER	☐ FOR	☒ IZO	☒ LYR
☐ NEO	☐ KOL	☐ EPO	☐ FMI	☐ BUD	
☐ CO	☐ PAR	☒ PER	☐ SQL	☐ TXP	

Positive allergens:

- Potassium dichromate 0.5 pet

- Nickelsulfate hexahydrate 5.0 pet

- Balsam Peru 25.0 pet

- Cl+Me-isothiazolinone (Kathon CG,100 ppm) 0.01

- Lyrall 5.0 pet

MOAH A index:

Occu.	Atopic.	Hand	Leg	Face
☐	☐	☐	☒	☐

Save

Clear

