Polysensitization in the Spanish Contact Dermatitis Registry (REIDAC): a 2019-2022 prospective study with cluster and network analysis

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ABSTRACT (228/300 words)

Background: There is still limited clinical-practice data on specific clinical and patch test features, as well as on allergen clusters in polysensitization (PS).

Objectives: To determine the frequency, relevance, symptoms duration and risk factors in polysensitized patients and to assess possible allergen aggregation.

Methods: Prospective multicentric study (January 2019 to December 2022) conducted in setting of the Spanish Contact Dermatitis Register (REIDAC). Clinical and patch test data of polysensitized and oligosensitized patients were compared and risk factors of PS were investigated with logistic multivariate regression. Unsupervised hierarchical clustering and network analysis were used to study allergen aggregation in PS.

Results: A total of 10,176 patients were analyzed. PS was found in 844 (8.3%). Current relevance was significantly higher in polysensitized patients ($p<0.01$). Risk factors for PS were atopic dermatitis (OR:1.58, 95%CI:1.24-2.02), age (≥60 years vs. ≤24 years, OR:1.75, 95%CI:1.25-2.44), and some special locations (legs vs. face OR: 1.54, 95%CI: 1.05-2.25, hands vs. face OR:1.46, 95%CI:1.15-1.85, arms vs. face OR:1.49, 95%CI:1.01-2.20, trunk vs. face OR: 1.40, 95%CI:1.06-1.85). Cluster and network analyses revealed specific allergen clusters and significant associations, including allergens belonging to metals group, fragrances and botanicals group, topical drugs group, rubber allergens, and biocides.

Conclusions: This study confirms that PS is structured by discernible patterns of specific-allergen clusters and reinforces significant allergen associations in PS. Cross-reactivity and/or concomitant sensitization could explain the formation of allergen clusters in PS.

BODY TEXT (2754/3000 words)

1. INTRODUCTION

Polysensitization (PS) is defined as the presence of three or more positive patch test reactions in baseline series.[1] It may also be referred to as "multiple contact allergies".[2] PS has been considered as a marker of susceptibility for skin sensitization.[3] Furthermore, it has been suggested that PS is the result of a myriad of biological circumstances, including the high exposure to allergens, the pre-existence of a dermatitis,[4] and/or genetic susceptibility.[5,6] However, the pathogenesis of the acquisition of multiple contact allergies is poorly understood. Several risk factors for PS have been proposed, including special locations of the dermatitis (hand,[7-9] feet,[9-11] leg,[8,10] axillae,[9] generalized dermatitis[8]), female gender,[4,11] occupational dermatitis,[4,11] age,[4,7,9] and atopic eczema.[11] Even if single allergens[8,12] or pairs of allergens (particularly of fragrances)[3] have been associated to PS, little data describes allergen clusters and their association in PS. A better knowledge of the associations seen in PS may lead to a better guidance on contact avoidance.

Polysensitization in patch testing cohorts has not been negligible (7.02-19.8%).[4,7] In clinical practice, PS could entail an impairment in quality of life, with more persistent dermatitis and relapses.[4,13] The relevance of patch reactions of PS patients has not been well characterised but could be probably more relevant in this group.[4] Disease duration in polysensitized patients has occasionally been linked to persistent and hard-to-treat eczema.[1,11]

The objectives of this study were to assess allergen aggregation in patients with polysensitization with unsupervised hierarchical cluster and network analysis, as well as to determine the frequency, relevance, symptoms duration and risk factors of PS in comparison to oligosensitized patients.

2. METHODS

A prospective multicentric study of the data included from January 2019 to December 2022. in the Spanish Contact Dermatitis Register (REIDAC) was conducted. Patients were patch tested with the 2019-2022 Spanish baseline. Allergens were commercially obtained from Chemotechnique (Vellinge, Sweden) and allergEAZE, SmartPractice (Calgary, Canada), depending on the availability of each center. Patch test performance

and readings (days 2, 4 and 7) were performed in accordance with the ESCD guidelines.[14] Relevance was evaluated after clinical examination and history of previous exposures. Current relevance was diagnosed in the case sensitization could explain or contribute to the dermatitis. REIDAC collects online data using the REDCap platform (http://www.project-redcap.org/ RRID:SCR_003445). Nature of the reaction (positive, negative, indetermined), its relevance (current, past, unknown), age, gender, occupational dermatitis (source of exposure in the work area or occupational setting), atopic dermatitis, affected site(s) (face, hands, arms, legs and trunk) and symptoms duration were collected. Oligosensitization (OS) was defined as positivity to one or two allergens of the Spanish baseline series, whereas PS was considered when three or more patch tests were observed. The registry was approved by the Complejo Hospitalario Universitario Insular-Materno Infantil Ethics Committee (2017/964) and its operation complies with the Declaration of Helsinki. All patients signed informed consent to participate.

2.1. Statistics

Continuous symmetrical variables are reported as means (standard deviations), continuous asymmetrical variables are reported as medians (interquartile range), and categorical variables are reported as absolute numbers (proportions). Significance was calculated with T-student and Chi-squared test. Factors associated with PS vs OS were investigated by logistic multivariate regression and expressed as odds ratios (OR) with 95% confidence intervals (95%CI). Unsupervised hierarchical cluster analysis was performed to create a dendrogram representation of the groups from polysensitized patients. Allergens were grouped according to its strength association, represented by the Pearson distance. A network analysis was performed in polysensitized patients to graphically represent these clusters and their interactions. Results were considered significant when the p-value was 0.05 or lower. All analyses were performed using STATA v.17.0 (Stata Corp. 2021. Stata Statistical Software: Release 17).

3. RESULTS

3.1. Polysensitization frequency, relevance, symptoms duration and factors risk for polysensitization

A total of 10,176 patients were patch tested with the Spanish baseline series. Table 1 and Table 2 present the demographic and clinical characteristics as well as the risk factors for

PS. In this period (January 2019-December 2022), a total of 4,469 patients (43.9%) presented with at least one positive result, 844 of whom (8.3%) were polysensitized and 3,625 (35.6%) were oligosensitized. A total of 7,981 positive patch test reactions were seen. In addition, 5,707 (56.1%) presented negative results. Statistically significant differences in baseline characteristics could be found for female sex ($p<0.001$) and age ($p<0.001$) between polysensitized and oligosensitized patients. The number of allergens found with current relevance was significantly higher in the group of PS ($p<0.01$). There were no differences in terms of median symptoms duration, with a median of 12.0 months for both groups.

Several possible risk factors for PS could be identified. Namely, age (≥ 60 years vs. ≤ 24 years, OR: 1.75, 95%CI: 1.25-2.44), atopic dermatitis (OR: 1.58 95%CI: 1.24-2.02), as well as some locations (legs vs. face (OR: 1.54 95%CI: 1.05-2.25), hand dermatitis vs. face dermatitis (OR:1.46 95%CI: 1.15-1.85), trunk dermatitis vs. face dermatitis (OR:1.40, 95%CI: 1.06-1.85), arm dermatitis vs. face dermatitis (OR:1.49, 95%CI: 1.01-2.20)), were associated with an increased risk of PS. In addition, female gender was associated with a risk reduction of PS (OR: 0.78, 95%CI: 0-65-0.94). Occupational dermatitis presented significant differences in the univariate analysis but did not result significant in the multivariate analysis.

3.2. Allergen clusters and cluster network

In terms of the analysis of single allergens, Table 3 presents the analyzed allergens of the Spanish baseline series with the number of patients with positive results. Nickel was the most common contact allergen for both groups. Polysensitized patients entailed a higher burden of positive results for all allergens.

In polysensitized patients, the total number of allergen combinations was of 577, with 466 unique allergen combinations that were exclusive of single patients. In contrast, the 20 most frequent combinations (3.5% of all combinations) (see Table 1S), were seen in 156 patients (18.5%). The most frequent combinations consisted of combinations of metals and biocides, metals and fragrances, multiple biocides and biocides and fragrances, among others. 453 patients (53.7%) presented sensitization to 3 allergens,

whereas sensitization to 4 (211, 25.0%) or ≥5 allergens (180, 21.3%) were also commonly found.

Unsupervised hierarchical cluster analysis revealed five different clusters. The dendrogram shows 4 heterogenous clusters that contain different allergen families (Clusters 1 to 4) and one cluster that is homogenous (Cluster 5). Cluster 1 (brown color) includes metals plus colophonium, lanolin alcohol and p-tert buthylphenol formaldehyde resin. Cluster 2 (green color) includes a myriad of allergens, mostly related to fragrances and allergens of topical products. Cluster 3 (light blue color) is composed of black dyes and caine mix, which is formed three local anesthetics of the caine group including benzocaine, tetracaine hydrochloride and dibucaine hydrochloride. Cluster 4 (dark blue color) includes non-formaldehyde biocides and rubber allergens. Cluster 5 (red color) includes formaldehyde and formaldehyde releasers. The exact distribution in clusters can be seen in Figure 1.

The dendrogram is complemented by a network analysis, which brings further information in regard to the association between clusters and specific allergens. In Cluster 1, metal allergens (nickel sulphate, cobalt chloride and potassium dichromate) are associated, with potassium dichromate associating also to p-tert butylphenol formaldehyde resin and neomycin sulphate (Cluster 2). Furthermore, the central area of Figure 2 shows the association of different allergens within Cluster 2 and with other Clusters. These allergens mostly belong to fragrances and botanical groups (colophonium, fragrance mix I, fragrance mix II, hydroxyisohexyl 3-cyclohexene carboxaldehyde (also known as Lyral), Myroxylon Pereirae oil and sesquiterpenic lactones), as well as topical drugs group (ethylenediamine dihydrochloride, budesonide, neomycin sulphate, tixocortol-21-pivalate and caine allergens). More specifically, the upper part of Cluster 2 shows the association of fragrance mix I, fragrance mix II and Lyral, and Myroxylon Pereirae oil, as well as their association(s) with other allergens, including allergens of Cluster 1 (colophonium and lanolin alcohol), and Cluster 2 (parabens, sesquiterpenic lactones, tixocortol pivalate and budesonide). The lower part of Cluster 2 shows the association of caine allergens (Cluster 3) with p-phenylenediamine as well as with several allergens of Cluster 2, including neomycin sulphate, budesonide, tixocortol-21-pivalate and sesquiterpenic lactones. In addition, ethylenediamine dihydrochloride is associated to many allergens of the same cluster (neomycin sulphate,

budesonide, sesquiterpenic lactones and epoxy resin). Methyldibromo glutaronitrile (Cluster 4) is not only associated to Cluster 2 associated allergens (Myroxylon Pereirae oil and sesquiterpenic lactones), but also to other non-formaldehyde biocides (methylisothiazolinone and methylchloroisothiazolinone) within the same cluster. Cluster 4 is also defined by the association of thiurams, carbamates, and mercapto compounds. Finally, methylisothiazolinone is associated to formaldehyde (Cluster 5), which is the main allergen of the formaldehyde and formaldehyde releasers cluster. All the significant allergen associations in the network analysis and their precise distribution can be seen in Figure 2.

4. DISCUSSION

PS remains as a relevant situation in Contact Dermatitis units. The frequency of polysensitized patients in this multicentric study is in line with previous findings,[4,7] even if patients' demographic baseline has proved to be different, particularly in terms of an increased presence of men. Current relevance of patch test positivity was higher in PS, confirming previous hypotheses. No differences for symptoms duration could be found between oligo and polysensitized patients, even if it has been suggested that PS could be related to long-lasting eczema.[1,11] This investigation has not studied the quality of life in relation to the course of the disease, which could be an important factor to take into account associated to symptoms duration.

Among the potential risk factors, this study also reinforces the presence of atopic dermatitis, leg dermatitis, hand dermatitis, and belonging to an older age group as relevant for PS. All these factors, are related to either an increased skin barrier alteration or to an increased theoretical exposure to allergens. Skin barrier impairment, which can be found in different skin conditions including atopic dermatitis, has been considered as a crucial phenomenon in allergen sensitization,[15] even if the risk of sensitization among atopic patients may be paradoxical in the setting of Th2-type immune responses.[16] Hand dermatitis has also been associated to PS,[17] possibly due to the high interaction of hands with many allergens. A similar rationale may be applied to the fact that PS is directly associated with a cumulative lifetime exposure.[4] In contrast with previous research, female gender and occupational dermatitis failed to be identified as significant risk-increasing factors.

It has been suggested that the acquisition of multiple contact allergens could appear to occur at random (non-clustered).[18,19] In contrast, it has recently been considered that PS would be structured by certain clusters of allergens.[9] Results from cluster and network analyses disclose that the association of allergens in PS is not at random, as well as the presence of patterns with allergen-specific clusters. The pathogenesis of PS remains widely unknown, but it is widely accepted that it is the result of a multifactorial process that includes environmental exposures, genetic susceptibility, plus the combination of pre-existing dermatoses.[1-4]

In terms of previously reported combination of allergens, combinations of metals, combinations fragrances and the combination of metals and fragrances were among the most commonly described.[20] In addition, previous studies have revealed significant allergen associations between metals of baseline series,[20-22] formaldehyde and formaldehyde releasers,[23] fragrances and metals,[21,23] fragrances and botanicals (e.g., fragrance mix I and Myroxylon Pereirae oil),[24] rubber allergens groups (carbamates and thiurams,[25] and the mercapto group[26]), some topical drugs allergens (e.g, ethylenediamine and neomycin),[25] and p-phenylenediamine and caine allergens.[25,27] Cluster analysis has aggregated metals, formaldehyde and its releasers, fragrances and botanicals, rubber allergens, ethylenediamine and neomycin, and p-phenylenediamine and caine allergens, reinforcing the relevance of these associations. Furthermore, these previous findings have been confirmed in the network analysis, with new associations being described (e.g., methyldibromo glutaronitrile with Myroxylon Pereirae oil and sesquiterpenic lactones).

In terms of the resulting clustering and network analysis of allergen associations, there is rationale to explain many of the specific association patterns found. In regard to allergen association, contact sensitization models have tried to explain these relationships mostly due to cross-reactivity, concomitant sensitization, simultaneous sensitization, or a combination of concomitant sensitization and cross-reactivity.[3,21,22,28]

In Cluster 1, the combination of metals (nickel sulfate, cobalt chloride and potassium dichromate) could be explained by cross-reactivity and concomitant sensitization to exposures that contain several metals.[4] Furthermore, this has been confirmed in real clinical practice by the observation of high metal co-reactions in nickel-sensitized patients.[29] The associations of potassium dichromate with both neomycin and p-tert butylphenol formaldehyde resin do not seem to be explained by concomitant

sensitization, nor do those correspond to cross-reactivity. We hypothesize that those could be multiple independent sensitizations.

The analysis of Cluster 2, may provide more evidence in terms of the relevance of environmental exposures in the process of PS. Allergens that belong to botanicals and fragrances groups may occur together, or in different sources but within a common setting. Perfumes and perfumed goods may contain several of these allergens,[30,31] pointing towards concomitant sensitization. The clinical relevance of the associations in the superior part of Cluster 2 may be important, particularly when colophonium, lanolin alcohol, Myroxylon Pereirae oil, and parabens are frequent in cosmetics and personal care products,[32] and have also been described among the most common sensitizers in patients with leg ulcers, accompanied by fragrances.[33-39] Despite lactones have been suggested to cross-react with colophonium due to the structural presence of similar terpenes,[40] this study has evidenced a strong association only with Fragrance mix II and Lyral. The lower part of this Cluster 2 describes the association of allergens that belong to topical steroids, topical antibiotics and topical anesthetics, the combination of which occurs within many different clinical scenarios (leg ulcers, atopic patients, skin infections, etc). Therefore, concomitant sensitization or simultaneous sensitization, could explain this relevant association of topical drug allergens in clinical practice. However, there are some strong associations within this area that may be more difficult to explain, such as the association epoxy resins with sesquiterpenic lactones, tixocortol pivalate or ethyelenediamine dihydrochloride. In this regard, we hypothesize that this may be due to multiple independent sensitizations.

Cluster 3 could be explained due to cross-reactivity between p-phenylenediamine and caine allergens, as well as between p-phenylenediamine and IPPD. Cross-reactivity for these allergens is related to the amine group in their benzene ring at the para position in these compounds.[41-44]

In Cluster 4, cross-reactivity between methylisothiazolinone and methylchloroisothiazolinone has been judged as possible, although it may be unlikely from a chemical perspective.[45] Therefore, it is believed that concomitant sensitization would explain the relationship between these two allergens.[46] In addition, methyldibromoglutaronitrile is associated to Myroxylon Pereirae oil and sesquiterpenic

lactones. Despite the use of the former allergen is banned in cosmetics, it may still be used in different products, including paints, adhesives, pharmaceutical products, domestic or hobby products, as well as cosmetics bought outside the EU.[47,48] In this regard, this association could hypothetically be indicative of independent, concomitant sensitizations. Cluster 4 is composed of rubber allergens, which may occur together in a material.[49] Bidirectional cross-reactivity between carbamates and thiurams has been described.[50] In addition, cross reactions between mercapto allergens and carbamates,[51] as well as concomitant reactions between mercapto compounds and thiuram have also been reported.[52] Therefore, this association is possibly explained by the combination of concomitant sensitization and cross-reactivity.

In Cluster 5, concomitant sensitization between formaldehyde and its releasers (mostly attributed to cosmetics) could explain the observed association.[53,54] Formaldehyde has cross reactivity with glutaraldehyde and glyoxal, but cross reactivity with formaldehyde releasers does not seem likely.[54]

4.1. Limitations and strengths

This research is part of a multi-centre REIDAC study with a large and recent sample of consecutive patients, which could be considered representative of the Spanish population. Patch testing has been performed in different Contact Dermatitis units, which may result in heterogeneity in the interpretation of patch test reactions.

5. CONCLUSION

At present, PS continues to be frequent in Contact Dermatitis Units. PS is associated with atopic dermatitis, particular anatomic locations (legs, hand, arm, trunk), and age. Positive patch test results in PS tend to have higher rates of current relevance. The use of clustering and network analysis reinforces previous cohort data and provides a stronger understanding of the formation of clusters in PS in clinical practice. Furthermore, concomitant sensitization and/or cross-reactivity could explain many of the associations of PS, highlighting the relevance of common exposures and/or similarity in the chemical structure of compounds in the acquisition of multiple contact allergies. The evaluation of causative products implicated in the etiology of the dermatitis may prove to be of interest in future studies since it may improve our understanding of the role of exposure(s) with single allergens and their aggregation in clusters. In conclusion, a better knowledge on

risk factors, cluster formation and mechanisms of PS could result in better allergen guidance and avoidance.

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TABLES

Table 1. Features of polysensitized patients, relevance of patch test reactions and risk factors

Variables	Polysensitization	Oligosensitization	p-value	Total
Year, n(%)				
2019	292 (8.5)	1296 (37.9)	-	1588 (46.5)[a]
2020	148 (8.8)	601 (35.5)	-	749 (44.3)[a]
2021	199 (8.3)	820 (34.2)	-	1019 (42.5)[a]
2022	205 (7.7)	908 (34.0)	-	1113 (41.7)[a]
Number of allergens, n(%)				
1	-	2515 (69.4)	-	-
2	-	1110 (30.6)	-	-
3	453 (53.7)	-	-	-
4	211 (25.0)	-	-	-
≥5	180 (21.3)	-	-	-
Relevance, n(%)*				
Current	1492 (46.0)	1823 (38.5)	<0.001	3315 (41.5)
Past	685 (21.1)	1625 (34.3)	<0.001	2310 (28.9)
Unknown	1069 (33.0)	1287 (27.2)	<0.001	2356 (29.5)

Abbreviations and notes: ([a]): calculated from the total number of patch tests of each year, (*): note that the number of relevant results is higher than the number of patients since each allergen is evaluated separately.

Table 2. Demographic features and risk factors of polysensitization (PS) vs oligosensitization (OS).

Demographics and clinical features					
Variables	Polysensitization	Oligosensitization	Multivariable logistic regression OR(95%CI)	p-value	Total
Duration in months, median (IQR)	12.0 (6.0-48.0)	12.0 (6.0-26.0)	-	0.128	12.0 (6.0-36.0)
Age, mean (SD)	51. 2 (17.0)	48.9 (17.0)	-	<0.01	49.4 (17.0)
Female sex	570 (67.5)	2783 (76.8)	0.78 (0.65-0.94)	0.01	3353 (75.0)
Occupational	197 (23.3)	716 (19.7)		<0.01*	913 (20.4)
AD	148 (17.5)	560 (15.4)	1.58 (1.24-2.02)	<0.01	708 (15.8)
Face dermatitis	150 (17.8)	841 (23.2)	1.00	-	991 (22.1)
Hand dermatitis	297 (35.1)	1161 (32.0)	1.46 (1.15-1.85)	<0.01	1458 (32.6)
Trunk dermatitis	139 (16.5)	514 (14.2)	1.40 (1.06-1.85)	0.018	653 (14.6)
Arm dermatitis	48 (5.7)	183 (5.0)	1.49 (1.01-2.20)	0.044	231 (5.2)
Leg dermatitis	56 (6.6)	171 (4.7)	1.54 (1.05-2.25)	0.027	227 (5.1)
≤ 24 years	70 (8.3)	337 (9.3)	1.00	-	407 (9.1)
25-59 years	509 (60.3)	2327 (64.2)		0.134*	2836 (63.5)
≥ 60 years	265 (31.4)	961 (26.5)	1.75 (1.25-2.44)	<0.01	1226 (27.4)

Abbreviations: AD (atopic dermatitis), CI (confidence interval), IQR (interquartile range), OR (odds ratio), SD (standard deviation). (*) p-values of univariate analysis. The OR of these variables have not been added due to non-significacy in the multivariate analysis.

Table 3. Distribution of allergens according to positivity in polysensitized (PS) and oligosensitized (OS) patients

PS	Positive, n(%)	OS	Positive, n(%)
Nickel sulphate	460 (54.5)	Nickel sulphate	1874 (51.7)
MI	305 (38.7)	MI	337 (10.2)
MI/MCI	282 (33.4)	MI/MCI	347 (9.6)
Fragrance mix I	216 (25.6)	Cobalt chloride	278 (7.7)
Cobalt chloride	213 (25.2)	p-phenylenediamine	253 (7.0)
Fragrance mix II	173 (22.0)	Fragrance mix I	206 (5.7)
Myroxylon Pereirae oil	166 (19.7)	Myroxylon Pereirae oil	164 (4.5)
Potassium dichromate	162 (19.2)	Fragrance mix II	148 (4.5)
Formaldehyde	151 (17.9)	Potassium dichromate	149 (4.1)
MDBGN	133 (16.0)	Formaldehyde	114 (3.1)
p-phenylenediamine	114 (13.5)	MDBGN	110 (3.1)
Thiuram mix	97 (11.5)	PTB-FR	97 (2.7)
Carba mix	92 (10.9)	Colophonium	81 (2.2)
Quaternium-15	65 (7.7)	Carba mix	69 (1.9)
Colophonium	57 (6.8)	Thiuram mix	61 (1.7)
PTB-FR	55 (6.5)	Ethylenediamine	57 (1.6)
Caine mix	50 (6.4)	Epoxy resin	56 (1.5)
Lyral	49 (6.3)	Caine mix	54 (1.5)
Ethylenediamine	48 (5.8)	Black rubber mix / IPPD	51 (1.5)
Neomycin sulphate	47 (5.6)	Neomycin sulphate	41 (1.1)
Epoxy resin	38 (4.5)	Budesonide	34 (0.9)
Budesonide	38 (4.5)	Lyral	28 (0.9)
Diazolidinyl urea	34 (4.0)	Lanolin alcohol	29 (0.8)

Imidazolidinyl urea	29 (3.4)	Quaternium-15	22 (0.6)
Lanolin alcohol	29 (3.4)	Imidazolidinyl urea	15 (0.4)
Paraben mix	29 (3.4)	Lactone mix (SL)	11 (0.3)
Mercapto mix	29 (3.4)	Paraben mix	12 (0.3)
Black rubber mix / IPPD	28 (3.3)	Diazolidinyl urea	11 (0.3)
2-mercaptobenzothiazole	27 (3.2)	Tixocortol-21-pivalate	10 (0.3)
Tixocortol-21-pivalate	20 (2.4)	Mercapto mix	7 (0.2)
Lactone mix (SL)	7 (0.90)	2-mercaptobenzothiazole	6 (0.2)
Phenoxyethanol	3 (0.50)	Phenoxyethanol	4 (0.1)

Abbrevations: MI/MCI: Methylisothiazolinone/methylchloroisothiazolinone, MI: Methylisothiazolinone, MDBGN: Methyldibromo glutaronitrile, PTB-FR: p-tert butylphenol formaldehyde resin, IPPD: N-Isopropyl-N'-phenyl-paraphenylenediamine, SL: sesquiterpenic lactones.

Figure legends

Figure 1. Unsupervised hierarchical clustering results

(Figure legend: none)

Figure 2. Network analysis: distribution of allergens in polysensitized patients

Figure legend: Allergens are represented as nodes and line connections, or edges, show statistically significant p-values, which stand for statistically probable coincidences and their significance depends on the frequency of positive reactions of the respective pair of allergens ($p<0.05$ for continuous lines, $p<0.01$ for discontinuous lines). This graph has unweighted and undirected edges. Ball size represents the frequency of positivity of each allergen.

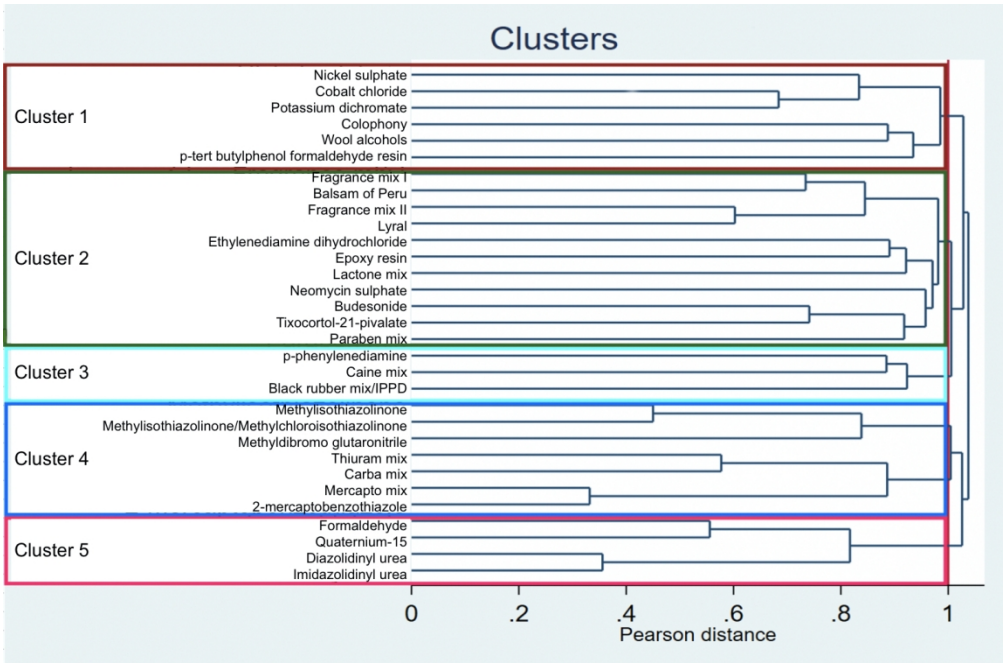
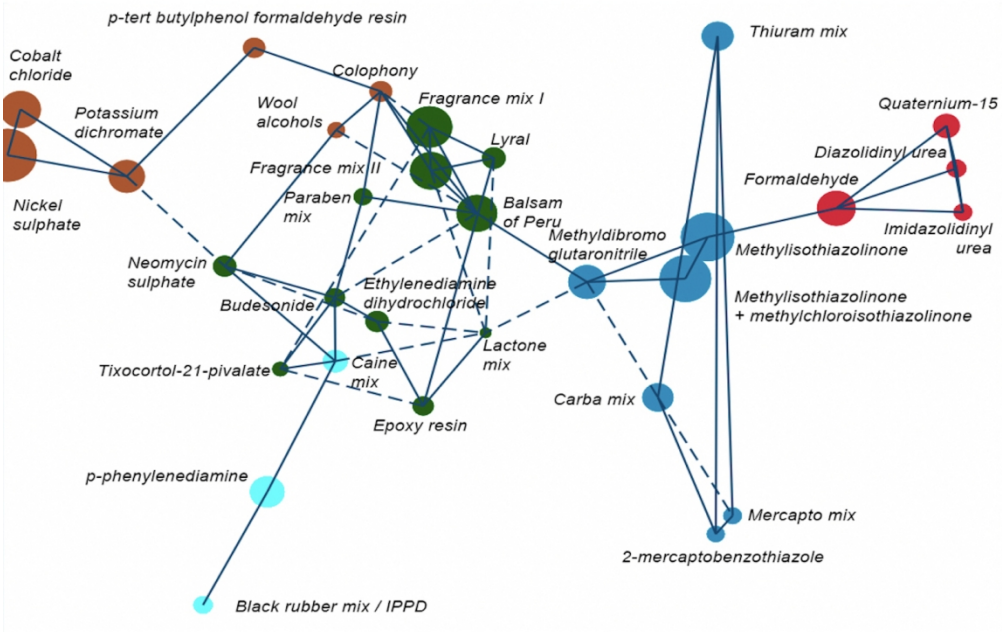


Figure 1. Unsupervised hierarchical clustering results

281x185mm (144 x 144 DPI)



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281x179mm (144 x 144 DPI)

Table 1S. Top 20 most frequent triple combination of allergens

Combination	N
Nickel sulphate – MI/MCI – MI	26
Nickel sulphate – Potassium dichromate – Cobalt chloride	24
MI/MCI – MDBGN – MI	9
Nickel sulphate – Cobalt chloride – p-phenylenediamine	9
Fragrance mix I – Balsam of Peru – Fragrance mix II	7
Nickel sulphate – Cobalt chloride – MI	7
Nickel sulphate – Fragrance mix I – Fragrance mix II	7
Nickel sulphate – Fragrance mix II – Lyral	6
Balsam of Peru – MI/MCI – MI	6
Fragrance mix I – MI/MCI – MI	6
Nickel sulphate – Caine mix – p-phenylenediamine	6
MI/MCI – p-phenylenediamine – MI	6
Nickel sulphate – MI/MCI – MDBGN – MI	5
Nickel sulphate – Carba mix – Thiuram mix	5
Nickel sulphate – Cobalt chloride – Thiuram mix	5
Nickel sulphate – Fragrance mix I – Balsam of Peru	5
Nickel sulphate – Cobalt chloride – MI/MCI	5
Nickel sulphate – MI/MCI – Formaldehyde – MI	4
Nickel sulphate – Potassium dichromate – Fragrance mix I	4
MCI/MI – Formaldehyde – MI	4

Abbrevations: MI/MCI: Methylisothiazolinone/methylchloroisothiazolinone, MI: Methylisothiazolinone, MDBGN: Methyldibromo glutaronitrile