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Article type : Letter to the Editor

To the editor,

Multiple tests are available to determine food and aero-allergen sensitization (1,2), but it is unclear if distinct sensitization patterns can be determined by using a combination of three testing modalities; skin prick test (SPT), specific IgE test and component resolved diagnostic tests to characterize or identify certain clinical phenotypes. This is a novel paper utilizing sensitization clusters (SPT, specific IgE tests and component resolved diagnostics) in an unselected cohort of 10/11 year-old children and its association with asthma, eczema, rhinitis and IgE-mediated food allergy. A birth cohort recruited in 2001 (Isle of Wight), was followed up prospectively to 10 years of age (3) (n = 827). At ten years SPT were performed to milk, egg, wheat pollen and flour, cod, sesame, peanut, house dust mite *Dermatophagoides pteronyssinus* (HDM), cat, grass and birch pollen (ALK-Abello, Hørsholm, Denmark) and lupin (Stallergens). A subset of children (n=246) consented to a blood test, analysed using ImmunoCap (ThermoFisher) using a predefined algorithm: Children were screened using the Fx5 test (milk, egg, wheat, cod, wheat, peanut, soy). Children with a positive screen (> 0.35 kuA/L), specific IgE to these foods were tested. We also tested specific IgE to lupin and sesame. If specific IgE to peanut and wheat were positive, then we tested: Wheat (rTria19, Wheat LTP, Gliadin) and peanut (Ara h1, Ara h2, Ara h3, Ara h8, Ara h9). For aero-allergens, Phadiatop Immunocap including grasses, trees, weeds, cat, dog, mites and molds was used. Those with a positive result were tested for specific IgE (grass, birch, house dust mite or dog, cat). If the specific IgE to grass or birch was positive, we tested for: Grass*:* Phlp1; Phlp7; Phlp p12; Phlp 5b and Birch (Bet v1 (PR-10); Bet v2 (profilin)). Rates of current allergic diseases were measured using the validated International Study of Asthma and Allergies in Childhood questionnaire at the same clinic

appointment (4). IgE mediated food allergy was defined as a positive food challenge or a positive SPT (≥3mm) and a convincing clinical history, as previously reported (3). Data was double entered in SPSS versions 20 and 21 and were compared and verified (SPSS Inc, Chicago, USA). Prevalence rates were computed together with 95% confidence intervals (CI). The CI were calculated using the exact CI, computed by the method of Clopper and Pearson. Chi square tests were undertaken to determine differences in those who did and did not consent for tests. Non-parametric cluster analyses were implemented, using the K-means approach. Standardized data were utilized in the analyses to reduce potential bias caused by different scales in the data. The scaling was conducted by standardizing with respect to mean (value-mean/standard deviation) for each variable. To determine the number of clusters, for each given number of clusters, we used the ratio between the sum of squares of variation between clusters and the sum of square of total variation. The final number of clusters is determined by maximizing the ratio with an effort to achieve parsimonious clusters. Logistic regressions, expressed as odds ratios (OR) were used to assess the association of hayfever, asthma, eczema, or food allergy with the pattern of allergic sensitization measures, IgE, and CRD. In this analysis, clusters with less than three subjects were deleted from further logistic regression analyses to avoid large uncertainty. Ethical approval for the study was obtained from the NRES South Central - Southampton B Research Ethics Committee (REF 10/H0504/11) and consent/assent obtained.

We followed 827, 85% of the original Food Allergy and Research study (FAIR) cohort at 10/11 years of age and of these 827; 246 children (29.75%; 246/827) consented to a blood test: 2.84% (7/246) had peanut allergy, 5.3% (12/246) had an IgE mediated food allergy, (of these 12 children diagnosed food allergy at 10 years of age, 7 children had a positive food challenge at 10 years, the other 5 children had a history of past positive food challenge/reaction on ingestion with sustained sensitization and declined the food challenge). 17.5% (43/246) reported asthma at 10 years, 30.1% reported eczema at 10

years and 37.8% (93/246) reported hayfever at 10 years. Sensitization data are summarized in table 1. Cluster analysis identified nine initial clusters, using 36 selected variables out of 39 in total that were tested. The following three variables were excluded due to missing values: milk SPT, lupin specific IgE and sesame specific IgE. In total, 4 participants having missing values in one or more of the 35 variables were excluded from cluster analyses. In addition, two clusters containing only 2 participants were excluded from analyses due to large uncertainty, leaving a total of 240 participants classified into seven discrete clusters. Cluster A had 167 participants, had the lowest rates of atopic disease and represents the reference group (see Figure 1). Cluster B (n=36) showed high values (i.e. larger wheal sizes) in terms of average wheal size only for grass SPT and was associated with hayfever (OR 2.7, p = 0.009, 95% CI 1.27 - 5.57), and asthma (OR 7.23, p < 0.001, 95% CI 3.097 - 16.914). Cluster C (n=8), showed high values in wheal size for wheat SPT, grass SPT, and wheat flour SPT, and larger measurements (i.e. higher specific IgE levels) for timothy specific IgE, birch specific IgE, cod specific IgE, soya specific IgE, Phlp 1 timothy, Phl p 5b timothy, Phl p 12 timothy and Bet v1 PR10 birch and was associated with hayfever (OR 4.4, p = 0.047, 95% CI 1.02 - 19.36). Cluster D (n=8), showed larger wheal sizes on average for sesame SPT, HDM SPT, and cat SPT, and large values for HDM specific IgE, peanut specific IgE, cod specific IgE, Ara H1 and Ara H2 and was associated with hayfever (OR 8.0, p = 0.013, 95% CI 1.55 - 41.1) and IgE mediated food allergy (OR 49.5, p = 0.001, 95% CI 6.71 - 365.11). For cluster E (n=8), larger wheal sizes for HDM SPT and birch SPT were observed and this cluster pattern was associated with hayfever (OR 8, p = 0.013, 95% CI 1.55 - 41.1), asthma (OR 6.08, p = 0.020, 95% CI 1.321 - 27.981) and IgE food allergy (OR 27.5, p = 0.002, 95% CI 3.29 - 229.68). Cluster F (n=7) had on average large wheal sizes for cod SPT, and large values for dog specific IgE, egg specific IgE and milk specific IgE and was not associated with any disease outcome. Cluster G (n=6) showed high values of wheal sizes on average for egg SPT, wheat SPT, fish SPT, peanut SPT, lupin SPT, and birch pollen SPT, and large values for Arah1, Arah3, Phl p 12 timothy and Bet v 2 profilin birch and was associated with asthma (OR 10.13, p < 0.0071, 95% CI 1.87 - 54.69) and IgE

mediated food allergy (OR 82.5, p < 0.001, 95% CI 9.87 - 689.06). Clusters D and E both had the highest rates of hayfever (75%) and eczema (37.5%). Cluster G had the highest rate of IgE mediated food allergy (50%) and asthma (50%).

Due to the uniqueness of our approach we are limited with the number of studies we can compare our data to. We have identified five studies that used similar, but not the same approach as our study. The Manchester Asthma and Allergy Study (MAAS) birth cohort (5), assessed sensitization status in children at the age of 11 years based on the ISAAC methodology and identified 3 distinct clusters in 11-year old children, showing distinct food and/or aero-allergen sensitization patterns (clusters) associated with hayfever or asthma. Garcia-Aymerich et al.(6), using specific IgE testing, identified two broad clusters in n= 17 209 children (at 4 and 8 years) from the MEDALL study: a symptomatic and a reference group, respectively characterized by high and low prevalence of allergic sensitization, morbidity, and multi morbidity defined as co-occurrence of asthma, hayfever and/or eczema. Amat et al.(7), using specific IgE testing, identified three clusters in n=271 6 year old children with eczema: cluster 1 showed low sensitization, cluster 2 had multiple sensitizations to food and aero-allergens and cluster 3 had moderate rates of sensitization to food allergens but no sensitization to aeroallergens. Mastrorilli et al.(8) used cluster analysis to identify five pollen food syndrome endotypes in Italian children aged 4-18 years. Finally, Cousin et al.(9), recently published, using SPT only, demonstrated using cluster analysis in n=317 peanut allergic children, that distinct clusters of possible cross reactions between tree nut, peanut and legumes exist; with those suffering from eczema showing the highest rates of cross-reactions between these three allergens.

The main limitation of our study is possible selection bias and the small number of participants in each cluster. Logistic regression shows wide CI: (e.g. cluster G and food allergy OR 82.5, 95% CI 9.87-689.06) which highlights the small numbers in some of the

clusters and the imprecision of the data. Although 71% of participants seen at 10 years consented to SPT, only 29% consented to a blood test. Although there were no differences in gender, maternal education or atopic family history between those who consented to SPT at one and ten years, there were some differences in those who did and did not consent to blood tests at age 10, namely the child's eczema ($p<0.001$) and hay fever status ($p<0.001$). These children all lived on the Isle of Wight, i.e the same demographic area. We compared being the first born child ($p=0.13$) and maternal diet during pregnancy (normal diet vs. vegan/vegetarian or other; ($p = 0.22$) and found no significant difference. There was however a significant difference in maternal education ($p=0.019$) with higher educated mothers (further or higher education) more likely to consent for blood test then those finishing/not finishing high school.

We have not tested for every possible available skin prick test, specific IgE test or component test, but limited our tests to those specified in the methods sections. To our knowledge, this is the first study to use cluster analysis based on SPT, specific IgE testing and CRD in an unselected population and measure their association with clinical outcomes. Although we could see some patterns of sensitization that clustered into groups of clinical disease as expected (e.g. aero-allergen sensitization associated with hay fever, or asthma and food allergen sensitization associated with IgE mediated food allergy), there was considerable overlap between clusters for allergic phenotypes. We wanted to determine if any particular pattern of clustering of sensitization levels using SPT, specific IgE or CRD tests adds to the clinical decision making process, however that does not seem to be the case in our study population. We suggest that better characterizing of sensitization clusters may in future aid in clinical diagnosis and risk stratification and indicate which tests for IgE detection may be most appropriate when dealing with certain clinical presentations.

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References

1. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. In: Journal of Allergy and Clinical Immunology. 2010. p. S1–58.
2. Treudler R, Simon J. Overview of component resolved diagnostics. Curr Allergy Asthma Rep. 2013;13(1):110–7.
3. Venter C, Patil V, Grundy J, Glasbey G, Twisleton R, Arshad SH, et al. Prevalence and cumulative incidence of food hypersensitivity in the first 10 years of life. Paediatr Allergy Immunol. 2016;27:452–8.
4. Von Mutius E. Epidemiology of asthma: ISAAC--International Study of Asthma and Allergies in Childhood. Pediatr Allergy Immunol. 1996;7(9 Suppl):54–6.
5. Simpson A, Lazic N, Belgrave DCM, Johnson P, Bishop C, Mills C, et al. Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years. Journal of Allergy and Clinical Immunology. 2014;
6. Garcia-Aymerich J, Benet M, Saeys Y, Pinart M, Basaga??a X, Smit HA, et al. Phenotyping asthma, rhinitis and eczema in MeDALL population-based birth cohorts: An allergic comorbidity cluster. Allergy Eur J Allergy Clin Immunol. 2015;70(8):973–84.
7. Amat F, Saint-Pierre P, Bourrat E, Nemni A, Couderc R, Boutmy-Deslandes E, et al. Early-onset atopic dermatitis in children: which are the phenotypes at risk of asthma? Results from the ORCA cohort. PLoS One [Internet]. 2015;10(6):e0131369. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4479437&tool=pmcentrez&rendertype=abstract
8. Mastrorilli C, Tripodi S, Caffarelli C, Perna S, Di Rienzo-Businco A, Sfika I, et al. Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification. Allergy Eur J Allergy Clin Immunol.

2016;71(8):1181–91.

9. Cousin M, Verdun S, Seynave M, Vilain AC, Lansiaux A, Decoster A, et al. Phenotypical characterization of peanut allergic children with differences in cross-allergy to tree nuts and other legumes. Pediatr Allergy Immunol. 2017;28(3):245–50.

Table 1: Sensitization rates in the first ten years of life

				Number of subjects sensitized based on SPT and Specific IgE n1 (SPT); n2 (Specific IgE)						
Sensitization	**Specific IgE tests (Measured by Fx5 and Phadiatop aero-allergen screen) n = 246**	**SPT N=246**	****p-values (Fisher's Exact tests for testing agreement with SPT results)**	**A (cluster size=167)**	**B (cluster size=36)**	**C (cluster size=8)**	**D (cluster size=8)**	**E (cluster size=8)**	**F (cluster size=7)**	**G (cluster size=6)**
	n (%; 95% CI)	**n (%; 95% CI)**								
Any of the predefined allergens	124 (50.4; 44.1-56.6)	88 (35.8; 29.8 – 441.82.1)	<0.001	18; 46	36; 36	7; 8	7; 8	8; 8	3; 7	6; 6
Any of the predefined food allergens	fx5 57* (23.2; 18.3-28.8)	58 (23.6; 18.3– 28.9)	<0.001	2; 14	29; 10	7; 7	5; 7	3; 2	2; 7	6; 5
Any of the predefined aero-allergens	113 (45.9; 39.7-52.1)	87 (35.4; 29.4 – 41.4)	<0.001	17; 38	36; 36	7; 8	7; 8	8; 8	3; 5	6; 5
Milk	14/57 (24.6; 13.4-35.8)	0	<0.001	0; 4	0; 0	0; 0	0; 2	0; 0	0; 5	0; 1
Egg	11/57 (19.3; 9.0-29.5)	1 (0.4; 0.0 – 1.2)	0.045	0; 4	0; 0	0; 2	0; 0	0; 1	0; 2	1; 1
Wheat	37/82* (45.1; 34.3-55.9) rTri a19: 3/40 (7.5;-0.7-15.7) Wheat LTP: 1/39 (2.6; -2.4-7.6) Gliadin: 1/39 (2.6; -2.4-7.6)	54 (22.0; 16.8 – 27.2) 1 (0.04 0.0 – 2.3)	<0.001	1; 1	29; 15	7; 8	3; 3	3; 1	2; 0	6; 5

Fish (Cod)	0/57 (0)	0	0.99	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0
Peanut	29/57 (50.9; 37.9-63.8) Ara h8: 6/33 (18.2; 5.0-31.3) Ara h1: 2/33 (6.1; -2.1-14.3) Ara h2: 6/33 (18.2; 5.0-31.3) Ara h3: 2/33 (6.1; - 2.1-14.3) Ara h9: 1/33 (3.03; -2.8-8.9)	10 (4.1;1.6 – 6.6)	<0.001	1; 1	0; 8	0; 7	2; 3	0; 1	0; 0	6; 6
Sesame	1/57 (1.8; -1.6-3.6)	1(0.4; 0.0 – 1.2)	0.99	0; 0	0; 0	0; 0	1; 1	0; 0	0; 0	0; 0
Lupin	3/57 (5.3;- 0.5 - 11.1)	3 (1.2; 0.3 – 2.6	0.99	0; 0	0; 0	0; 0	1; 1	0; 0	0; 0	2; 2
Soya (specific IgE only)	19/57 (33.3; 21.1-45.5)	-		---	---	---	---	---	---	---
House dust mite	HDM1 81/113 (71.7; 63.4-80.0) HDM2 75/113 (66.4; 57.7-75.1)	50 (20.3; 15.3 – 25.3)	<0.001	15; 29	18; 21	1; 7	6; 8	6; 7	1; 4	1; 3
Grass	Timothy 89/113 (78.8; 71.3-86.3) Phlp1 79/92 (85.9; 78.8-93.0) Phlp7 4/91 (4.4; 0.2-8.6) Phlp p12 14/91 (15.4; 7.9-22.8) Phlp 5b 47/91 (51.6; 41.3-61.9)	60 (24.4; 19.0 – 29.8)	<0.001	1; 18	33; 36	7; 8	6; 8	3; 5	2; 5	6; 5

Cat	49/113 (43.4; 34.3-52.5)	27 (10.8; 6.9 – 14.7)	<0.001	3; 8	14; 19	0; 3	4; 7	2; 4	1; 2	2; 3
Dog	42/113 (37.2; 28.3-46.1)		---	---	---	---	---	---	---	---
Birch IgE Betv1 Betv2	35/113 (31.0; 23.1 – 40.0) 15/39 (38.5; 24.9 – 54.1) 13/39 (333; 20.6 – 49.0))	12 (4.9; 2.2 – 7.6)		0; 4	0; 11	1; 5	1; 5	7; 7	0; 0	3; 4

CI: Confidence interval. LTP: Lipid transfer protein. HDM: House dust mite
Legend: *Specific IgE testing to a food was performed only in those with a positive Fx5; or in case of a negative Fx5, if they had a positive SPT to the food. The denominator for all the foods was 57, as n=57 had a positive FX5. In the case of wheat, n=25 had positive SPT to wheat despite a negative specific IgE, hence the denominator n=82. We only tested for the components if the specific IgE to a food was positive (>0.35 kA/L).
**Calculated on the assumption that if the fx5 and the SPT were both negative, then the Specific IgE would also be negative (so the denominator for both SPT and specific IgE is now n=246).

Figure 1 Selected summary characteristics of clusters

Figure 1