Biomarkers to predict changes in peanut allergy in children over time

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Abstract

Background: Various biomarkers are used to define peanut allergy (PA). We aimed to observe changes in PA resolution and persistence over time comparing biomarkers in PA and peanut sensitised but tolerant (PS) children in a population-based cohort. **Methods:** Participants were recruited from the EAT and EAT-On studies, conducted across England and Wales and were generally well exclusively breastfed babies recruited at 3 months old and followed up until 11 years old. Clinical characteristics, skin prick test (SPT), sIgE to peanut and peanut components and mast cell activation tests (MAT) were assessed at 12m, 36m and 7-11y. **Results:** The prevalence of PA was 2.1% with only 1 child having PA resolution at 7-11y. PA children had larger SPT size, higher peanut-sIgE, Ara h 2-sIgE and MAT (all p<0.001) compared to PS children at 36m and 7-11y. SPT, peanut-sIgE, Ara h 2-sIgE and MAT between children with persistent PA, new PA, outgrown PA and PS were statistically significant at both 36m and 7-11y (p<0.001). Those with persistent PA had SPT, peanut-sIgE and Ara h 2-sIgE that increased over time and MAT which was highest at 36m. New PA children had increased SPT and peanut-sIgE from 36m to 7-11y, but MAT remained low. PS children had low biomarkers across time. **Conclusions:** In this cohort, few children outgrow or develop new PA between 36m and 7-11y. Children with PA have significantly higher SPT, peanut-sIgE, Ara h 2-sIgE and MAT compared to PS children, evident from 12-36m of age.

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(*ISRCTN15837754*) and SOFTER (Clinicaltrials.gov: NCT03270566) trials as well as the UK-Irish Atopic eczema Systemic Therapy Register (A-STAR; ISRCTN11210918) and a Principle Investigator in the European Union (EU) Horizon 2020-funded BIOMAP Consortium (*http://www.biomap-imi.eu/*). He also leads the EU Trans-Foods consortium and directs the Global Atopic Dermatitis Atlas (www.atopicdermatitisatlas.org). His department has received funding from Sanofi-Genzyme and Pfizer for skin microbiome work. He has also received compensation from the British Journal of Dermatology (reviewer and Section Editor) and EuroGuiDerm (guidelines lead).

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Background:

The global prevalence of IgE-mediated peanut allergy (PA) is estimated to be between 0.2% to 4.5% depending on country (1), with the UK prevalence of PA in children being 2%. Approximately 20% of peanut allergic patients outgrow their allergy over time (2, 3) but using biomarkers to help predict which children this is more likely to occur in is less understood.

The gold standard to assess for diagnosis and possible resolution of food allergy is the oral food challenge (OFC). (4) However, this test comes with the risk of life-threatening anaphylaxis, is time-consuming, laborious, and costly, especially if multiple food challenges are needed to assess changes in allergic status. Other allergy tests, such as skin prick tests (SPT), specific-IgE (sIgE) to peanut and peanut components, are more

commonly used to help establish PA diagnosis. (5) They can also be used to understand resolution and, specifically, to help determine the right time to reintroduce an allergen back into the diet. More recently, the basophil activation test (BAT) and mast activation test (MAT) have been demonstrated to have clinical utility to support the diagnosis of food allergy. (6, 7) The MAT works by using LAD2 mast cells (a human mast cell line) which are sensitised with patient plasma or serum, stimulated with allergen (e.g. peanut) and CD63 expression is measured using flow cytometry. Both BAT and MAT have been shown to have high specificity (ranging between 96 and 100%) in diagnosing PA (7) but due to practical reasons, is primarily available in the research setting.

Food allergies, such as egg and cow's milk allergies, are commonly outgrown (8, 9) but for peanut and tree nut/sesame allergies, this occurs less frequently. Decreasing SPT wheal size and levels of allergen-sIgE over time are suggestive of food allergy resolution. (10) However, the use of MAT in the context of allergy persistence or resolution has not yet been investigated.

The aims of this study were to assess the utility of different diagnostic biomarkers to characterize different trajectories of PA and PS in a general population of children over the span of a decade. We compared results between peanut allergic and peanut sensitised but tolerant children across different time points to better understand their use in predicting PA persistence or resolution over time.

Methods:

Study population

Participants were selected from the EAT and EAT-On studies and informed consent was obtained. (11) The EAT study reviewed the children at 3 months (3m), 12 months (12m), and 36 months (36m) of age with the primary outcome being the diagnosis of IgE mediated allergy between 1 and 3 years of age. The EAT-On study was the follow-on study conducted to establish whether the effects seen at the end of the EAT study represented a delay in food allergy onset or sustained tolerance. The EAT-On cohort was seen between ages 7-11 years old.

Allergic status was confirmed by either a positive OFC or based on clinician-taken history of reaction and SPT>5mm if an OFC was not conducted. Tolerance was determined by a negative OFC and/or consumption of peanut regularly in the child's diet as defined by the EAT-On study protocol (i.e. at least 3g of peanut protein 3 times in the last 6 months). If the child was not consuming peanut and OFC was indeterminate or not available, in the presence of SPT=0 mm they were also considered not allergic.

Skin prick tests (SPT)

SPT were performed to peanut and aeroallergens on the forearm or back, using a standardized lancet (ALK-Abello), peanut extract (ALK-Abello), histamine 10 mg/mL or 50% glycerol, 50% buffered saline. Skin test sites were measured after 15 minutes as the average of the widest diameter and perpendicular of the wheal.

Blood collection

Serum sIgE and IgG4 to peanut and peanut components (rAra h 1, rAra h 2, rAra h 3, rAra h 8) were determined by ImmunoCAP (Thermofisher, Uppsala, Sweden). Peanut component sIgE was assayed for participants with a peanut IgE [?]0.1 kUA/L; for subjects with levels <0.1KU/L, an imputation was performed. A ratio of Ara h 1, h2 and h3 to peanut sIgE was calculated to examine the distribution of each component in relation to sIgE to peanut. The median of this ratio was used to determine the imputed value for each Ara h component for patients who had peanut sIgE<0.1kUA/L. The ratio of peanut-specific IgG_4:IgE was calculated after peanut-specific IgG_4 levels were converted from μg/L to ng/mL and peanut sIgE levels were converted from kU/L to ng/mL using the formula ($IgG_4 \div (IgE \times 2.4)$). (12) Plasma or serum was also used for MAT.

Peanut Oral food challenge (OFC)

An OFC was offered to any participant who had:

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- SPT > 0 mm to peanut;
- Previous history of peanut allergy and SPT[?]5mm;
- Previous history of peanut allergy whose testing suggested they might have outgrown it (i.e. SPT < 5 mm or negative SPT)
- Participants who were infrequent consumers of peanut (i.e. who consumed less than 3 grams of peanut protein at least 3 times in the last 6 months).

All OFCs were open challenges unless there was investigator concern about subjective symptoms, in which case a double blinded placebo-controlled food challenge was performed. The open challenges involved a single-dose cumulative challenge or 6-7 dose incremental challenge if deemed to be high risk.

Mast cell activation test (MAT)

For the MAT, patients with peanut-sIgE [?]1.0 kUA/L were selected given previously reported lower threshold of peanut-sIgE to induce mast cell activation which can vary with intrinsic mast cell reactivity. (7) MAT was performed as previously reported. (7, 13) LAD2 cells (Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases) were primed with IL-4 and incubated for 5 days before being sensitized with patient serum. The cells were stimulated with peanut extract (ALK Abello) diluted in RPMI medium (GIBCO, Paisley, UK) at two concentrations (1000ng/mL and 10,000ng/mL), anti-IgE (1ug/mL, Sigma-Aldrich, Poole, UK) and ionomycin (1 ug/mL, Millipore). Cells were stained with CD63-allophycocyanin (Biolegend, San Diego, Calif) and surface markers IgE-PE (Biolegend), CD32-APC, FceRI-FITC (eBioscience, San Diego, Calif) before viability dye eFluor 450 (eBioscience) was added. Flow cytometry (CytoFLEX flow cytometer) was performed with FACSDiva software (BD Biosciences, San Jose, Calif) and data was analysed using FlowJo[TM] v10.8 Software (BD Life Sciences, Ashland OR). MATs were performed for peanut allergic and peanut sensitised but not allergic children (PS) as defined above at the three time points (12m, 36m, 7-11y), for which samples were available.

Statistical analysis

Data analysis was performed using Stata Statistical Software Release 17 College Stations, TX. StataCorp LLC. Mann Whitney and Kruskall-Wallis tests were performed to compare clinical characteristics and biomarkers between peanut allergic and PS children and for sub-group analysis. Logistic regression models were also used to determine if any covariates predicted PA status at 7-11 years. Univariate analysis was performed to look at covariates affecting PA at 7-11y followed by multivariable regression models to compare all biomarkers at each time point (i.e. SPT, peanut sIgE, Ara h 2-sIgE, MAT at 12 months) and another to look at longitudinal comparison of single biomarkers at all 3 time points (i.e. SPT at 12m, 36m, 7-11y) in predicting PA status at 7-11y.

Results:

Study population

A total of 947 patients were enrolled in the EAT-On study. Each child's PA status was determined as shown in Supplementary Appendix Figure 1. One child was excluded as they were on peanut oral immunotherapy at the 7-11y time point and 2 children with likely persistent PA had a telephone visit only, so no blood samples were collected. Therefore, 48 children fit the criteria for the sub-group analysis (Supplementary Appendix Figure 2).

The prevalence rate of PA in the EAT-On cohort at 7-11y was 2.1% (20/947) which is similar to the EAT end of study prevalence rate of 1.9% (22/1189). For the children who had clinical assessments during the EAT and EAT-On studies, the rate of PA resolution was 5.5% (1/18). If we were to include the 2 children who only had a telephone visit with confirmed PA by parental report during EAT-On and the 2 children who were PA during EAT but did not return for EAT-On but assume they were still PA, the rate of resolution would be 4.5% (1/22).

The initial analysis compared the PA (n=20) and PS (28) groups. Eighteen children who were PA at 36m

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were still allergic at 7-11y (persistent PA), 2 were not PA at 36m but PA at 7-11y (new PA), 1 was PA at 36m but no longer allergic at 7-11y (outgrown PA), and 27 were peanut sensitised at 36m but never allergic at any time point (NA).

The PA group were significantly more likely to have asthma at 7-11y old compared to the PS group (45% vs 17.9%, p<0.05) – Supplementary Appendix Table S3. There were no significant differences between age, sex, ethnicity, history of eczema, eczema at 7-11y or rhinitis at 7-11y.

Comparing biomarkers between peanut allergic and peanut sensitised tolerant groups

PA children had significantly higher peanut SPT than PS children at 12m, 36m and 7-11y (Table 1). Median peanut-sIgE levels were also significantly different between PA vs PS groups at 36m and 7-11y. This was further reflected in the peanut component data with the PA group having significantly higher Ara h 1-sIgE, Ara h 2-sIgE and Ara h 6-sIgE levels at the 7-11y time point compared to the PS group. Interestingly, at 36m of age, the PA group already had higher Ara h 2-sIgE levels compared to the PS group (4.0kUA/L vs 0.04kUA/L, p<0.001) and this increased and remained significantly higher as they got older (16.3kUA/L vs 0.06kUA/L, p<0.001 at 7-11y). The %CD63-positive LAD2 cells following peanut stimulation was higher in the PA group at the 3-year and 7-11year time points compared to the PS group.

Table 1: Comparison of biomarkers across time between Peanut Allergic vs Peanut sensitized not allergic patients with allergic status determined at 7-11 years. Mast cell activation following stimulation with 1000ng/ml of peanut extract.

	Peanut allergic (n=20)* Median (IQR)	Peanut sensitised but not allergic (n=28)* Median (IQR)	P value
Peanut extract SPT mm 3 months** 12 months 36 months 7-11 year	0 (0, 0) (n=5) 4.5 (3, 7.5) (n=18) 8.5 (6.3, 10.3) 9.3 (7.3, 10.3)	0 (0, 2) (n=11) 1 (0, 4.8) 1.5 (0, 5) 0 (0, 2)	0.20 **0.02 <0.001** **<0.001**
Peanut sIgE kUA/L 3 months 12 months 36 months 7-11 years	0.05 (0.03, 0.2) (n=18) 1.4 (0.64, 6.5) (n=16) 9.3 (1.6, 46.7) (n=19) 24.8 (3.6, 85.2)	0.06 (0.03, 0.2) (n=25) 1.5 (1.1, 5.4) (n=27) 0.8 (0.2, 1.5) 0.9 (0.1, 5.2) (n=27)	0.8 0.7 **<0.001** **<0.001**
Peanut components-sIgE kUA/L (3 months) Ara h 1 Ara h 2 Ara h 3 Ara h 8	(n=18) 0.07 (0, 0.4) 0.01 (0.01, 2.0) 0.02 (0.002, 0.04) 0.003 (0.002, 0.005)	(n=25) 0.1 (0, 0.2) 0.005 (0.002, 0.01) 0.01 (0.005, 0.02) 0.003 (0.001, 0.005)	1.00 0.11 0.67 0.73
Peanut components-sIgE kUA/L (12 months) Ara h 1 Ara h 2 Ara h 3 Ara h 8	(n=16) 0.4 (0.05, 4.0) 0.6 (0.07, 6.7) 0.06 (0.02, 0.3) 1.5 (0.03, 10.3)	(n=27) 0.2 (0.02, 0.7) 0.2 (0.02, 1.2) 0.1 (0.03, 0.5) 0.004 (0.001, 0.013)	0.81 0.39 0.49 0.24
Peanut components-sIgE kUA/L (36 months) Ara h 1 Ara h 2 Ara h 3 Ara h 8	(n=19) 0.1 (0.04, 1.8) 4.0 (1.3, 28.3) 0.03 (0.02, 0.4) 1.5 (0.03, 10.3)	0.1 (0.03, 0.2) 0.04 (0.02, 0.2) 0.1 (0.06, 0.2) 0.04 (0.01, 0.5)	0.38 **<0.001** 0.72 0.14

	Peanut allergic (n=20)* Median (IQR)	Peanut sensitised but not allergic (n=28)* Median (IQR)	P value
Peanut components-sIgE kUA/L (7-11 years) Ara h 1 Ara h 2 Ara h 3 Ara h 6 Ara h 8	1.8 (0.12, 28.4) 16.3 (1.9, 54.5) 0.28 (0.02, 3.5) 10.4 (1.6, 31.9) 1.5 (0.03, 10.3)	(n=27) 0.06 (0.01, 0.2) 0.06 (0.03, 0.2) 0.06 (0.03, 0.2) 0.06 (0.03, 0.1) 0.26 (0.06, 0.6)	**<0.001 <0.001** **<0.01 <0.001** 0.14
Peanut sIgG4 g/L 3 months 12 months 36 months 7-11 years	11.3 (0, 40.1) (n=17) 202.8 (74.6, 433.6) (n=16) 407.4 (175.7,1526.3) (n=18) 736.2 (210.9,1482.4)	7.3 (0, 27.3) (n=22) 78.3 (34.2, 506.9) (n=26) 353.4 (96.4, 1882.8) 523.7 (124.5,2673.3) (n=27)	0.54 0.69 0.51 0.88
Peanut sIgG4:sIgE ratios 3 months 12 months 36 months 7-11years	9.2 (0, 211.1) (n=17) 35.9 (7.1, 166.8) (n=16) 19 (8.6, 51.2) (n=12) 11.3 (5.1, 35.1)	15 (0, 326.2) (n=22) 15.1 (4.7, 296.4) (n=26) 191.4 (39.6, 2493.6) (n=19) 544 (30.4, 2448) (n=27)	0.95 0.70 **<0.01** **<0.001**
MAT to peanut (%CD63 LAD2 cells) 3 months 12 months 36 months 7-11 years	10.7% (8.7-12.7) (n=2) 0.7% (-0.34, 11.6) (n=15) 11.5% (1.3, 30.2) 12.2% (5.0, 35.8)	– 0.32% (-0.1, 7.8) (n=25) 0.19% (-0.2, 0.6) (n=26) 0.3% (0.1, 0.8) (n=27)	– 0.31 **p<0.001** **p<0.001**
*The number of patients in the groups differ based on sample availability and are denoted separately. **At the 3m time point, only children in the early introduction group had SPT performed and very few had sufficient bloods for MAT to be performed.	*The number of patients in the groups differ based on sample availability and are denoted separately. **At the 3m time point, only children in the early introduction group had SPT performed and very few had sufficient bloods for MAT to be performed.	*The number of patients in the groups differ based on sample availability and are denoted separately. **At the 3m time point, only children in the early introduction group had SPT performed and very few had sufficient bloods for MAT to be performed.	*The number of patients in the groups differ based on sample availability and are denoted separately. **At the 3m time point, only children in the early introduction group had SPT performed and very few had sufficient bloods for MAT to be performed.

Comparing biomarkers between sub-groups of peanut allergic status at the 7-11 y time point

Further sub-group analyses were performed to assess changes in PA over time (Table 2).

Table 2: Biomarkers in participants grouped according to peanut allergic status at 7-11 years of age. Median and interquartile range are indicated. Mast cell activation following stimulation with 1000ng/ml of peanut extract.

Peanut allergic status at the 7-11y time point

	Persistent peanut allergy (n=18)	New peanut allergy (n=2)	Outgrown peanut allergy (n=1)	No peanut allergy (n=27)	P value
Peanut SPT mm (IQR)	Peanut SPT mm (IQR)	Peanut SPT mm (IQR)	Peanut SPT mm (IQR)	Peanut SPT mm (IQR)	Peanut SPT mm (IQR)

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3 months* 12 months 36 months 7-11 years	0 (0, 0) 5.8 (3.5, 8) 8.8 (7, 10.5) 9.3 (7.5, 10.5)	0 (0, 0) 1 (0, 2) 1.3 (0, 2.5) 7 (4, 10)	– 5.5 8.0 0	0 (0, 2) 1 (0, 4.5) 1 (0, 5) 0 (0, 2)	– **0.02** **<0.001** **<0.001**
Peanut specific IgE (kUA/L) 3 months 12 months 36 months 7-11 years	Peanut specific IgE (kUA/L) 0.05 (0.0, 0.3) 1.5 (0.9, 7.2) 9.5 (6.1, 46.7) 30.0 (4.2, 85.4)	Peanut specific IgE (kUA/L) 0.03 (0.03, 0.03) 0.4 (0.1, 0.8) 0.7 (0.2, 1.3) 11.7 (2.9, 20.5)	Peanut specific IgE (kUA/L) 0.08 1.3 0.01 0.02	Peanut specific IgE (kUA/L) 0.05 (0.0, 0.3) 1.6 (1.1, 5.4) 0.9 (0.2, 1.5) 0.6 (0.1, 4.9)	Peanut specific IgE (kUA/L) 0.41 0.28 **<0.001** **<0.001**
Peanut components specific IgE (kUA/L) 3 months Ara h 1 Ara h 2 Ara h 3 Ara h 8	Peanut components specific IgE (kUA/L) 0.07 (0, 0.4) 0.01 (0.01, 2.0) 0.02 (0.002, 0.04) 0.003 (0.002, 0.005)	Peanut components specific IgE (kUA/L) 0.01 (0.01, 0.01) 0.01 (0.01, 0.01) 0.01 (0.01, 0.01) 0.01 (0.01, 0.01)	Peanut components specific IgE (kUA/L) 0.01 0.01 0.01 0.01	Peanut components specific IgE (kUA/L) 0.01 (0.01, 0.01) 0.01 (0.01, 0.01) 0.01 (0.01, 0.01) 0.01 (0.01, 0.01)	Peanut components specific IgE (kUA/L) 0.91 0.93 0.87 0.99
12 months Ara h 1 Ara h 2 Ara h 3 Ara h 8	0.3 (0.05, 4.0) 0.8 (0.07, 6.7) 0.08 (0.02, 0.3) 0.001 (0, 0.01)	0.3 (0.01, 0.6) 0.1 (0.01, 0.1) 0.02 (0.01, 0.03) 0.01 (0.01, 0.01)	0.06 1.7 0.01 0.01	0.2 (0.02, 0.6) 0.1 (0.01, 0.7) 0.1 (0.03, 0.4) 0.01 (0.01, 0.01)	0.47 0.13 0.26 0.92
36 months Ara h 1 Ara h 2 Ara h 3 Ara h 8	0.04, 2.4) 9.3 (1.4, 31.9) 0.05 (0.02, 0.6) 0.01 (0, 0.01)	0.04 (0.02, 0.1) 0.03 (0.01, 0.04) 0.03 (0.03, 0.03) 0.05 (0.01, 0.09)	0.01 0.01 0.01 0.01	0.1 (0.03, 0.2) 0.04 (0.01, 0.2) 0.1 (0.05, 0.2) 0.04 (0.01, 0.05)	0.21 **<0.001** 0.31 0.28
7-11 years Ara h 1 Ara h 2 Ara h 3 Ara h 6 Ara h 8	2.0 (0.1, 35.7) 19.8 (1.9, 60.5) 0.3 (0.01, 3.9) 13.0 (2.3, 32.4) 0.5 (0, 7.9)	1.3 (0.3, 2.4) 6.4 (0.1, 12.8) 0.19 (0.2, 0.3) 1.5 (0.05, 2.9) 48.7 (2.6, 94.9)	0.01 0.01 0.01 0.01 0.01	0.02 (0.01, 0.1) 0.04 (0.01, 0.1) 0.04 (0.01, 0.1) 0.04 (0.01, 0.1) 0.1 (0.01, 0.6)	**<0.001** **<0.001** 0.05 **<0.001** 0.16
Peanut-specific IgG4 and specific IgG4:IgE Ratios Peanut sIgG4 (g/L) 3 months 12 months 36 months 7-11 years	Peanut-specific IgG4 and specific IgG4:IgE Ratios 6.1 (0, 52.8) 202.9 (102, 436.8) 494.2 (215.9, 1800.6) 736.2 (217.2, 1361.3)	Peanut-specific IgG4 and specific IgG4:IgE Ratios 15.2 (15.2,15.3) 233.7 (37.1, 430.3) 121.1 (23.3, 218.8) 840.9 (840.9, 1613.4)	Peanut-specific IgG4 and specific IgG4:IgE Ratios - - 99.3 44.3	Peanut-specific IgG4 and specific IgG4:IgE Ratios 8.4 (0, 27.3) 84.8 (34.5, 506.9) 386.5 (96, 2171.5) 719 (127.9, 2685)	Peanut-specific IgG4 and specific IgG4:IgE Ratios 0.66 0.37 0.35 0.43

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Peanut specific IgG4:IgE ratios 3 months 12 months 36 months 7-11 years	4.4 (0, 63.6) 30.4 (6.7, 78.2) 14.7 (7.5, 21.1) 10.4 (4.5, 37.4)	211.7 (211.1, 212.4) 213.2 (193.5, 232.9) 60.6 (51, 70.1) 21.3 (9.8, 32.8)	- - 4135.9 1044.8	20.5 (0, 326.2) 17.9 (6.1, 296.4) 175.8 (39.6, 1222.7) 483 (30.4, 2448.0)	0.39 0.22 **0.03** **<0.01**
Mast cell activation to peanut (%CD63+ LAD2 cells) 12 months 36 months 7-11 years	Mast cell activation to peanut (%CD63+ LAD2 cells) 4.6 (0.16, 25.2) 17.8 (3.1, 30.3) 12.7 (7.6, 38.9)	Mast cell activation to peanut (%CD63+ LAD2 cells) 0.1 (0.01, 0.2) 0.3 (0.3, 0.4) 0.2 (0.1, 0.4)	Mast cell activation to peanut (%CD63+ LAD2 cells) – 0.01 0.5	Mast cell activation to peanut (%CD63+ LAD2 cells) 0.3 (0.01, 3.3) 0.2 (0.01, 0.6) 0.2 (0.01, 0.8)	Mast cell activation to peanut (%CD63+ LAD2 cells) 0.20 **<0.001** **<0.001**
At the 3m time point, only children in the early introduction group had SPT performed.	*At the 3m time point, only children in the early introduction group had SPT performed.	*At the 3m time point, only children in the early introduction group had SPT performed.	*At the 3m time point, only children in the early introduction group had SPT performed.	*At the 3m time point, only children in the early introduction group had SPT performed.	*At the 3m time point, only children in the early introduction group had SPT performed.

There were statistically significant differences in SPT, peanut-sIgE, peanut Ara h2-sIgE component and CD63+ LAD2 cells activation between the 4 groups at both 36m and 7-11y (p<0.001) – Figure 1, Figure 2, Figure 3 and Figure 4. Over time, children with persistent PA had SPT and peanut-sIgE and Ara h 2-sIgE levels that were suggestive of PA as early as 12m of age that increased and remained persistently high; however, MAT was highest at the 36m time point. New PA children showed increasing SPT, peanut-sIgE and Ara h 2-sIgE over time but the levels increased slower over time compared to those with persistent PA. The MAT in this group also remained low at all time points. The new PA children also had an increase of Ara h8-sIgE from 36m to 7-11y although this was not significant. The time at which this increase occurred suggests that these children developed their PA at some point between 36m to 7-11y of age. Both children had peanut introduced early into their diet with reports of both consuming peanut at 12m. However, at 36m one was no longer consuming peanut but the other was and at 7-11y, both were no longer consuming peanut and their biomarkers were consistent with PA. The child who outgrew PA had raised SPT and peanut-sIgE levels at 12m of age which was consistent with PA diagnosis. However, by 36m, although SPT was still high, their peanut-sIgE levels had already started to decrease and by 7-11y both SPT and peanut-sIgE were low. In this child, MAT remained low at all time points. Children who were NA had consistently low biomarkers across time in keeping with what would be expected of non-allergic children. There were significant differences in SPT between the groups at the 12m, 36m and 7-11y time points and total sIgE at the 36m and 7-11y time points. Specifically, Ara h 2-sIgE was already significantly higher (p<0.001) in persistent PA group at 12m of age (9.3kUA/L) and increased to 19.8kUA/L by 7-11y of age. This differs to the new PA group who at 12m had undetectable Ara h 2-sIgE (0.03kUA/L) that then increased by 7-11y (6.4kUA/L) which is when they were diagnosed with PA. MAT was significantly different between the groups with higher mast cell activation occurring in persistent PA group at the 36m and 7-11y time points.
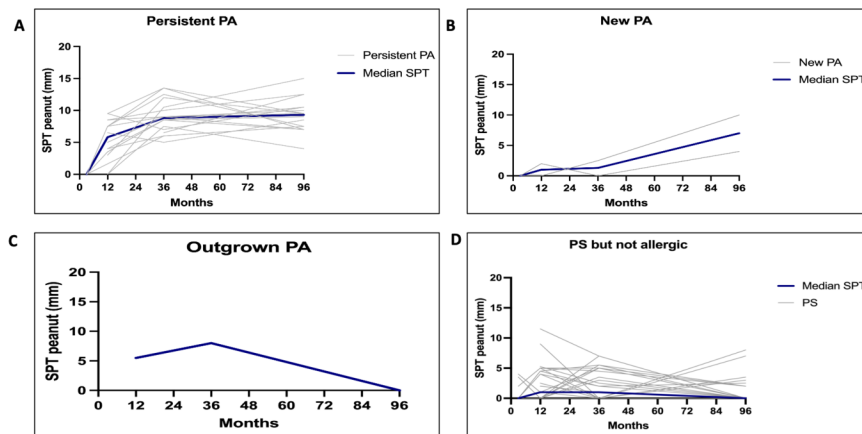
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Figure 1: Changes in peanut SPT across time in A) Persistent PA, B) New PA, C) Outgrown PA, D) Peanut sensitized but never allergic (NA). The grey lines represent individual patients and dark blue line represents the median SPT at each of the time points.
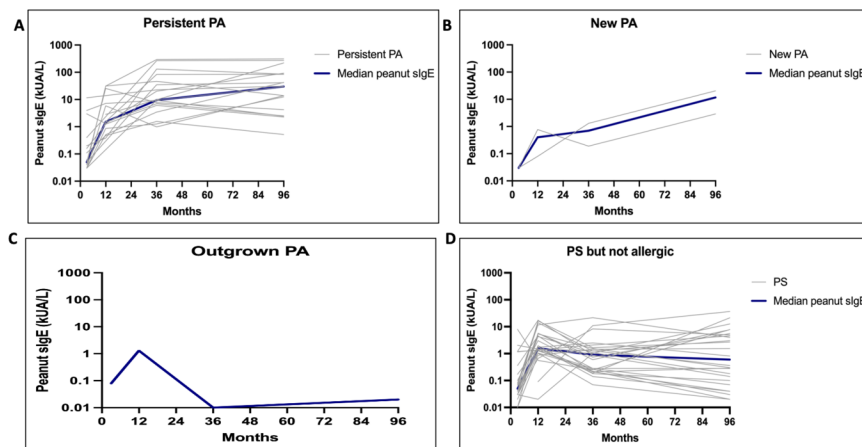

Figure 2: Changes in individual peanut-specific IgE across time in A) Persistent PA, B) New PA, C) Outgrown PA, D) Peanut sensitized but never allergic (NA). The grey lines represent individual patients, and the dark blue line represents the median sIgE at each of the time points. IgE levels are represented on a log 10 scale.
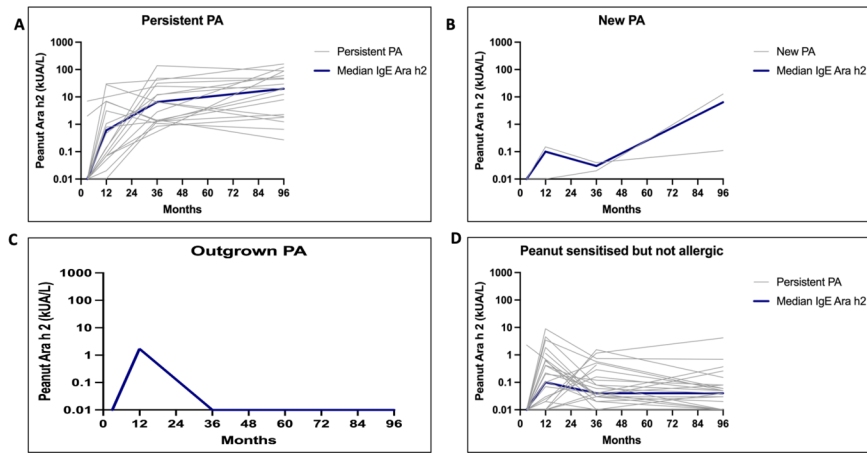
Figure 3: Changes in individual Ara h 2-specific IgE across time in A) Persistent PA, B) New PA, C) Outgrown PA, D) Peanut sensitized but never allergic (NA). The grey lines represent individual patients and he dark blue line represents the median sIgE Ara h 2 at each of the time points. sIgE levels are represented on a log 10 scale.
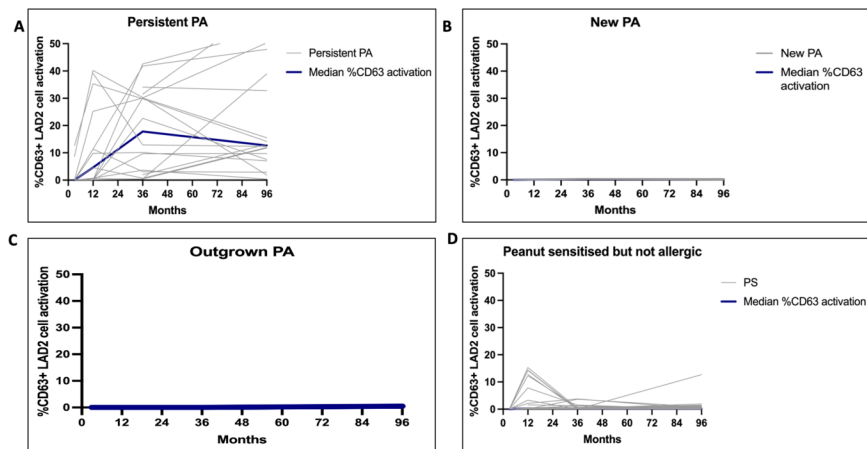

Figure 4: Changes in individual mast cell activation across time in A) Persistent PA, B) New PA, C) Outgrown PA, D) Peanut sensitized but never allergic. The grey lines represent individual patients and the dark blue line represents the median %CD63+ LAD2 cells at each of the time points.

Comparing IgG$_4$:IgE ratios between PA and PS groups

Comparison of peanut IgG4 and IgG4:IgE ratios between PA and PS children revealed significantly higher peanut-specific IgG$_4$:IgE ratios in the PS group compared to the PA group at 36m of age (191.4 vs 19, p<0.01) and at the 7-11y time point (544 vs 11.3, p<0.001), respectively (Table 2). This was also reflected in the subgroup analysis, with the children who did not have PA because they outgrew it or never had it, as they had higher peanut-specific IgG4:IgE ratios compared to those who had persistent or new PA at both the 36m (p<0.05) and 7-11y (p<0.01) time points.

Biomarkers associated with peanut allergy at 7-11 years

Logistic regression analyses was used to determine if any covariates were found to be related to developing PA at 7-11y. Demographic and clinical characteristics such as age, sex, ethnicity or history of AD were not

significantly associated with PA at 7-11y. Univariate analyses were performed and the following covariates were found to be significant: childhood asthma at 7-11y, SPT to peanut at 12m, 36m and 7-11y, peanut-sIgE at 3y and 7-11y, Ara h 2-sIgE at 3y and MAT to peanut at 3y and 7-11y (Supplementary Appendix Table S4).

With the multivariable regression models, when comparing all biomarkers at each of the time points, only peanut SPT at 7-11y was associated with increased odds of having PA at 7-11y (coeff 0.72, 95% CI 0.1, 1.3) (Supplementary Appendix Table S5). When looking at the model that looked at individual biomarkers across time, only SPT, specifically at the 7-11y time point was significantly associated with PA status at 7-11y (coeff 0.9, 95% CI 0.2, 1.6). Small numbers of children who developed new PA or resolved their PA prevented longitudinal analyses.

Discussion:

A good understanding of the different trajectories of PA over time is important to safely diagnose and manage PA. The prevalence of PA at both the end of EAT and 7-11y was relatively stable with 2.1% at 7-11y and 1.9% at the end of EAT. There were two new cases of PA that developed after 36m and only 1 child outgrew PA by 7-11y. Children with persistent PA at 7-11y had significantly higher levels of SPT, peanut-sIgE, Ara h 2-sIgE and mast cell activation compared to children who were PS, with many biomarkers being diagnostic of PA by 36m.

At 12m of age the persistent PA children already had median SPT and Ara h 2-sIgE levels consistent with a PA diagnosis which only continued to increase over time. Studies have reported SPT of [?]6mm and Ara h 2-sIgE between 0.1-3kUA/L being predictors of persistent. (14, 15) Ara h 2-sIgE and Ara h 6-sIgE are the peanut components most indicative of true peanut allergy (16), which was consistent with our findings at the 7-11y time-point. The IgG_4:IgE ratios were significantly lower in the PA group and specifically in the children with persistent PA at 36m and 7-11y. Overall, the MAT was suggestive of PA at the 36m and 7-11y time points in the children who had persistent PA at 7-11y. There were only two persistent PA patients who had plasma available from their 3m EAT study visit who had MAT performed (Table 2). Their median CD63 activation was 10.7% which is suggestive of PA at such an early age. The higher MAT at these time points reflected the higher levels of peanut-sIgE levels, which we know from previous work induces greater mast cell activation (7). These changes in biomarkers demonstrate that biomarkers that are high early in childhood and increase over time are indicative of persistent PA.

There were only 2 patients who developed new peanut allergy. Their SPT and peanut-sIgE biomarkers were initially low until 36m but increased over time so that by 7-11y they were consistent with a PA diagnosis. They were consuming peanut in early childhood but by 7-11y had stopped all peanut consumption which supports previous evidence that shows that patients who do not consume peanut regularly after a negative OFC are at higher risk of recurrent PA, which may have contributed to why these patients developed new PA. (17) Interestingly, MAT remained negative across time in these two children even at the 7-11y time point which differed from the children with persistent PA who had higher MAT at 36m and 7-11y. A possible explanation for this lower MAT in new PA is the quality of the IgE. Hemmings et al showed that IgE functional characteristics modify mast cell activation with higher mast cell activation resulting from higher peanut-sIgE levels, higher specific activity, higher diversity and higher avidity of IgE for peanut (18). It is possible that for those who had new PA later in childhood, the allergic immune response was not fully developed and sIgE had lower levels, specific activity, diversity and avidity for peanut allergens.

There was only 1 child that outgrew their PA by 7-11y confirmed by negative OFC. Although we cannot infer conclusions on trends in biomarkers overtime based on their results alone, the patterns observed were still interesting. This child had SPT and Ara h 2-sIgE suggestive of PA at 12m of age; although the SPT remained high at 36m, Ara h 2-sIgE level was negative. Their peanut-sIgE level was lower at all time points with a peak of 1.3kUA/L at 12m and then was negative by 36m and remained so until 7-11y. The IgG_4:IgE ratio was also very high at the 36m and 7-11y time points which would be consistent with tolerance to peanut as seen in previous studies (12).

This study is unique in that it looks at the changes in PA in a population-based cohort of children over the span of a decade. The longitudinal nature of this study and the availability of biomarkers at the different time points helps to explain how PA is largely stable in later childhood. Our data demonstrates that high biomarkers in early childhood are associated with PA persistence which is consistent with previous findings (15). MAT has high specificity in identifying children who will clinically react to peanut (19) but this is the first study looking at MAT over time and for those with persistent PA, levels were raised by 12m. The utility of MAT was limited in children with very low levels of peanut-sIgE, like those who developed or resolved their PA.

The major limitation of this study is the small number of children in the sub-group analysis. Only 2 children developed new PA and 1 child outgrew PA which makes it difficult to draw conclusions. We had hoped to compare biomarkers predicting resolution of PA with persistence of PA but this was not possible in this cohort. There was also missing biomarker data in terms of baseline SPT (i.e. these were not performed for children randomised to the standard introduction group) and Ara h component-sIgE data (i.e. was only performed if peanut sIgE>0.1kUA/L). We were able to impute the component data based on sIgE levels but there were still some children who did not have data available. Also, as the children were all recruited from the EAT-On Study, definitions for allergic status and tolerance were based on the study protocol to allow for consistency in the data analysis. In an ideal setting, all children selected for the biomarker work would have had OFC to confirm their PA status at 7-11y of age.

To conclude, the rate of PA in this cohort of children was 2.1% at 7-11y. Children with PA at 36m and 7-11y have significantly higher SPT, peanut-sIgE, Ara h2-sIgE and MAT compared to PS children. These biomarkers are already raised between 12m and 36m of age. For those that develop new PA or outgrow their PA, the timing at which this happens likely occurred between 36m and 7-11 years of age but small numbers and low biomarkers prevented additional conclusions.

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