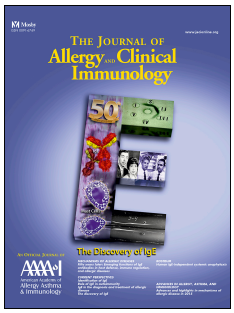
CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in children with peanut allergy
Authors	Hourihane, Jonathan O'B.;Allen, Katrina J.;Shreffler, Wayne G.;Dunn Galvin, Gillian;Nordlee, Julie A.;Zurzolo, Giovanni A.;Dunn Galvin, Audrey;Gurrin, Lyle C.;Baumert, Joseph L.;Taylor, Steve L.
Publication date	2017-02-24
Original Citation	Hourihane, J. O. B., Allen, K. J., Shreffler, W. G., Dunngalvin, G., Nordlee, J. A., Zurzolo, G. A., Dunngalvin, A., Gurrin, L. C., Baumert, J. L. and Taylor, S. L. (2017) 'Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in children with peanut allergy', Journal of Allergy and Clinical Immunology, 139(5), pp. 1583-1590. doi:10.1016/j.jaci.2017.01.030
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.jaci.2017.01.030
Rights	© 2017 American Academy of Allergy, Asthma & Immunology. Published by Elsevier. This manuscript version is made available under the CC-BY-NC-ND 4.0 license. - http://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2026-11-15 12:44:21
Item downloaded from	https://hdl.handle.net/10468/3982

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Accepted Manuscript



Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in peanut allergic children

Jonathan O'B. Hourihane, MD, DM, Katrina J. Allen, MD, PhD, Wayne G. Shreffler, MD, PhD, Gillian Dunngalvin, PhD, Julie A. Nordlee, MS, Giovanni A. Zurzolo, PhD, Audrey DunnGalvin, PhD, Lyle C. Gurrin, PhD, Joseph L. Baumert, PhD, Steve L. Taylor, PhD

PII: S0091-6749(17)30324-X

DOI: 10.1016/j.jaci.2017.01.030

Reference: YMAI 12664

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 29 August 2016

Revised Date: 22 December 2016

Accepted Date: 9 January 2017

Please cite this article as: Hourihane JO'B, Allen KJ, Shreffler WG, Dunngalvin G, Nordlee JA, Zurzolo GA, DunnGalvin A, Gurrin LC, Baumert JL, Taylor SL, Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in peanut allergic children, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.01.030.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in peanut allergic children

Jonathan O'B Hourihane MD, DM[1] J.Hourihane@ucc.ie

Corresponding author
Paediatrics and Child Health, University College Cork, Ireland
Tel +353214901237
Fax +353214345217

Katrina J Allen MD, PhD[2, 3] mailto:Katie.allen@rch.org.au

Wayne G Shreffler, MD, PhD[4] mailto:wshreffler@mgh.harvard.edu

Gillian Dunngalvin PhD[1,5] 109331239@umail.ucc.ie

Julie A Nordlee, MS[6] jnordlee1@unl.edu

Giovanni A Zurzolo PhD[2,7] Giovanni.zurzolo@live.vu.edu.au

Audrey DunnGalvin PhD[1,5] A.DunnGalvin@ucc.ie

Lyle C. Gurrin PhD [8] lgurrin@unimelb.edu.au

Joseph L Baumert PhD[6] jbaumert2@unl.edu

Steve L TaylorPhD [6] staylor2@unl.edu

[1]*Paediatrics and Child Health, University College, Cork, Ireland*
[2] *Murdoch Childrens Research Institute, Department of Paediatrics The University of Melbourne, Melbourne, Australia*
[3] *Department of Allergy and Immunology, Royal Children's Hospital, Melbourne, Australia*
[4] *Food Allergy Centre and the Centre for Immunology and Inflammatory Disease, Massachusetts General Hospital / Harvard Medical School, Boston, USA*
[5] *School of Applied Psychology, University College, Cork, Ireland*
[6] *Food Allergy Research and Resource Program, University of Nebraska.*
[7] *Centre for Chronic Disease, College of Health and Biomedicine, Victoria University, Melbourne, Australia*
[8] *Melbourne School of Population and Global Health, University of Melbourne*

Funding
Food Allergy Research & Resource Program

ACCEPTED MANUSCRIPT

Abbreviations

DBPCFC	Double blind, placebo-controlled food challenge
ED	Eliciting dose
FAQLQ	Food allergy related quality of life questionnaire
FEV_1	Forced expiratory volume in 1 second
LOAEL	Lowest adverse effect level
OFC	Oral food challenge
PA	Peanut allergy
PAL	Precautionary allergen labelling
PATS	Peanut allergen threshold study
spIgE	Specific IgE
SPT	Skin prick test
VITAL	Voluntary Incidental Trace Allergen Labelling
VSEP	VITAL scientific expert panel

Capsule summary

The derived ED_{05} for peanut (1.5mg peanut protein) was given in a single dose to 378 peanut allergic subjects. Only 8 subjects (2.1%) met predetermined criteria for an objective reaction, suggesting the derived ED_{05} could be used as a safe reference dose.

Clinical Implications

The ED_{05} for peanut (1.5mg peanut protein) was validated in a multicentre study, using a novel single dose challenge design, which provides a significant quality of life benefit for parents of participants and could be adapted to other research or clinical settings.

Keywords

Eliciting dose (ED), Food Allergy related Quality of Life Questionnaires-(FAQLQ), Single dose, Peanut thresholds**,** Oral Food Challenges (OFC), Voluntary Incidental Trace Allergen Labelling (VITAL). Peanut Allergen Threshold Study (PATS)

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Abstract

Background

Eliciting doses (ED) of allergenic foods can be defined by the distribution of threshold doses for individuals within a specific population. ED_{05} is the dose that elicits a reaction in 5% of allergic subjects. The predicted ED_{05} for peanut (PN) is 1.5 mg of peanut protein (6 mg whole peanut).

Objective

We sought to validate the predicted peanut ED_{05} (1.5 mg) with a novel single dose challenge.

Methods

Consecutive eligible peanut allergic children in 3 centres were prospectively invited to participate, irrespective of previous reaction severity. Predetermined criteria for objective reactions were used to identify ED_{05} single dose reactors.

Results

518 children (mean age 6.8 years) were eligible. No significant demographic or clinical differences were identified between 381(74%) participants and 137 (26%) non-participants or between subjects recruited at each centre. 378 children (206 male) completed the study. Almost half the group reported ignoring precautionary allergen labelling. 245 (65%) experienced no reaction to the single dose of peanut. 67 (18%) reported a subjective reaction without objective findings. 58 (15%) experienced signs of a mild and transient nature that did not meet the pre-determined criteria. Only 8 subjects (2.1%, 95% CI 0.6%-3.4%) met the pre-determined criteria for an objective and likely related event. No child experienced more than a mild reaction, 4 of the 8 received oral antihistamines only and none received epinephrine. Food allergy related quality of life improved from baseline to 1 month post challenge regardless of outcome (eta squared = 0.2, $p < 0.0001$). Peanut SPT, peanut and Ara h 2 spIgE levels were not associated with objective reactivity to PN ED_{05}.

ACCEPTED MANUSCRIPT

105 **Conclusion**

106 A single administration of 1.5 mg PN protein elicited objective reactions in fewer than the predicted
107 5% of peanut-allergic subjects. The novel single dose OFC appears clinically safe and patient-
108 acceptable, regardless of the outcome. It identifies the most highly dose-sensitive food allergic
109 population, not otherwise identifiable using routinely available peanut SPT or spIgE levels but this
110 single-dose approach has not yet been validated for risk assessment of individual patients.

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Introduction

Food allergic individuals are clinically selected to participate in diagnostic or research oral food challenge (OFC) protocols that use graded, incremental doses administered at short, fixed time intervals. Subjects who have experienced anaphylaxis are often not offered routine clinical OFC and may be excluded from research OFC protocols (1). It is generally not possible from graded protocols to determine whether a reaction has occurred to a *discrete* threshold dose of the allergenic food or alternatively has been the result of the *cumulative* dose consumed by the allergic individual at the time of reaction.

The eliciting dose (ED) for a peanut allergic reaction in 5% of the peanut allergic population (ED_{05}) has been estimated at 1.5 mg of peanut protein (6 mg of whole peanut) based upon the population distribution of threshold doses (children and adults) from graded, blinded oral challenges of 750 peanut allergic individuals (2-4).

This study aims to assess the precision of the predicted ED_{05} using a single dose challenge (6 mg peanut = 1.5mg of peanut protein, approximately $1/100^{th}$ of a peanut kernel) in an unselected group of peanut-allergic children and to validate the processes used to develop the only existing reference doses for peanut, which have been based upon the ED_{01} (which is the dose which elicits reactions in 1% of subjects studied) (2). It is likely that subjects who react only mildly at the ED_{05} would tolerate the ED_{01} at least as well (4). This may assist clinicians, regulators and other stakeholders in risk management for peanut allergic subjects.

ACCEPTED MANUSCRIPT

Methods

We have already published an in-depth description of the background and methodology of the PATS study (please see reference 5). Additional details are provided below.

Recruitment

This multi-centre study involved three geographically diverse teaching centres, set in University-affiliated hospitals, providing local, regional and national allergy services. To minimise recruitment bias, the protocol required that the study was discussed fully with every potentially suitable child and family, met during routine medical encounters in clinic or hospital attendances. Families who chose not to participate were asked to complete a study-specific "non-participant" questionnaire, adapted from Osborne et al (6) and to give written informed consent for their routinely available laboratory data to be examined anonymously in the study. Inclusion and exclusion criteria are shown in Table 1.

Food Allergy related Quality of Life Questionnaires-(FAQLQ)

Validated FAQL-Parental form (FAQL-PF) and FAQL Child form (FAQL-CF) questionnaires were self-administered prior to OFC (T1) and 1 month after OFC (T2) to assess the impact of this novel single dose OFC protocol on FAQL (8). FAQL-PF and CF are age appropriate questionnaires that assess the health related quality of life (HRQL) of children with food allergy. The PF version is completed by a parent of the food allergic child (0-12 years) and the CF by the child themselves (8-12 years) on a 7-point scale ranging from not at all (1) to extremely (7). It has been found to have excellent reliability ($\alpha > 0.9$), and construct, cross-cultural, content and longitudinal validity. A higher score on either questionnaire reflects higher burden and poorer FAQL. A lower score reflects lower burden/better FAQL.

ACCEPTED MANUSCRIPT

Single dose Oral Food Challenge (OFC)

The shelf-stable single-dose challenge cookies were manufactured at University of Nebraska-Lincoln, USA and then distributed to participating clinic centres. Peanut content was determined using the Neogen ® Veratox ® Quantitative Peanut Allergen Test (Neogen Corporation, Lansing MI). This assay was also used to establish a validated mixing method to achieve a homogeneous incorporation of peanut flour into the formulation as well as determining that all ingredients in the formulation were below the limit of quantitation (2.5 ppm). The stability of product was established by meeting acceptable criteria for water activity and microbial load. To maintain taste and texture cookies were stored frozen until use. The single-dose cookie (6 mg whole peanut = 1.5 mg peanut protein) consisted of granulated sugar, brown sugar, all-purpose wheat flour, vegetable shortening, salt, baking soda and light roast, partially defatted peanut flour (Golden Peanut Company, Alpharetta, Georgia USA). The cookie was eaten under standard open OFC conditions in hospital. For subjects allergic to other cookie ingredients (e.g. wheat), the peanut dose of 1.5 mg peanut protein was administered as the same light roast, partially defatted peanut flour in a vehicle food of the subject's choice. Routine OFC monitoring was performed, according to local clinical practice. Children were observed until 2 hours after OFC if no symptoms and signs were elicited or until 2 hours after such symptoms and signs had resolved, with or without treatment.

Criteria for a positive OFC result.

A highly liberal, inclusive strategy was used to capture clinical data during the OFC. Staff were encouraged to make extensive notes, recording *any* physical or behavioural changes observed or self-reported during the single-dose OFC. Predetermined objective criteria were used because the ED_{05} was predicted on the basis of challenge-associated objective responses only (1-4). The prior agreed upon objective criteria for a positive OFC result occurring within 2 hours of ingestion were:

ACCEPTED MANUSCRIPT

3 or more concurrent noncontact urticaria persisting for at least 5 minutes; or perioral or periorbital angioedema; or rhinoconjunctivitis including sneezing; or diarrhoea; or vomiting (excluding gag reflex); or anaphylaxis (with evidence of circulatory or respiratory compromise, e.g. persistent cough, wheeze, change in voice, stridor, difficulty breathing, and collapse) (9).

Subjective symptoms were also recorded, such as: palatal itch, headache, dizziness, bloating, abdominal pain, cramps, muscle aches, aching joints, anxiety, tension, and agitation.

Case definition

When the clinical study was completed all co-investigators met in person and reviewed all clinical comments written by staff in each centre during the study. The above criteria were applied and cases were designated “objective” or “subjective” and then as having met or not met the predetermined objective criteria as above.

Blood test

A blood sample was taken for peanut spIgE component analysis (local hospital laboratories, using ThermoFisher Immunocaps®, according to manufacturer’s instructions) and quantitative peanut-specific IgE fluoroenzyme immunoassays 20 minutes after OFC.

Sample size estimation

Assuming that the observed proportion of the sample that react to the single dose OFC is 5%, a sample size of 375 corresponds to a 95% confidence interval for the population proportion with a lower limit of 3.1% and an upper limit of 7.8% using the properties of the binomial distribution. The investigators felt that this degree of precision in estimation was sufficient to rule out gross incompatibility between the predicted and observed proportion of participants reacting to the single dose.

ACCEPTED MANUSCRIPT

Statistics

Data were analysed using SPSS Version 22(IBM, Evanston, Illinois, USA). Two sample t-tests for continuously valued variables and Pearson's chi- square test or Fisher's exact test (for low prevalences) for binary variables were conducted to determine the extent of any covariate imbalance between participants and non-participants. Differences in means and proportions between centres were also examined using similar statistical methods. The impact of the single dose protocol on FAQL was analysed using multivariable regression analysis.

Partial Eta-squared' (η_p^2), also known as 'R-Squared', was the effect size produced by the statistical tests used in this study. There are many advantages to including effect size when reporting significant results. Effect size is not influenced by sample size or number of variables. While a significant result (p value) shows whether an effect exists, it does not reflect the size of the effect. Therefore both the magnitude (effect size) and significance (*P* value) are essential results to be reported (10-12). A small effect is less than 0.08, a medium effect is less than 0.24 and a large effect size is 0.25 and above (11).

Ethical approval

This Study was approved by Cork University Hospital Research Ethics Committee (ECM 4 g), Melbourne Royal Children's Hospital Human Research Ethics Committee (HRECApp 32166A), and the Partners Human Research Committee (2012P002475). Written, informed parental and adolescent consent and younger children's assent (according to local IRB age-related requirements) were obtained.

Results

ACCEPTED MANUSCRIPT

Between October 2013 and February 2015, 518 patients were serially approached for participation (Figure). One hundred thirty-seven individuals were deemed either ineligible or did not wish to take part in the study. Three hundred seventy-eight completed the challenge protocol. Three subjects did not complete the protocol. Comparisons of participants and non-participants in each centre are shown in Table 2. Univariate analysis of variance showed no significant age differences between participants and non-participants (p = 0.62), controlling for centre location (p=0.84,). Sixty percent of the overall sample was male. Twenty-two percent of females approached did not participate, compared to 30% of males (x^2= 6.7, p=0.035). There was no difference in participant sex between centres (x^2= 2.6, p=0.63).

A significant association was found between entry criteria and study centre location. Twenty-seven percent of Irish subjects had been diagnosed with peanut allergy by the most stringent criterion (positive OFC), compared to 11% in Australia and only 2.5% in the US, (p<0.001). However, diagnostic method did not significantly differ between participants and non-participants (x^2= 3.6, p=0.17) or between sexes (x^2= 6.17, p=0.19).

Reactions to single dose ED_{05} OFC

381 participants took part in this stage of the study; two were excluded due to incomplete ingestion of the peanut cookie. One subject was excluded before starting the protocol due to inter-current illness, evident on clinical examination on the day of study. 378 subjects completed the protocol. 362 subjects (96%) received the single dose in the cookie. The remaining 16 subjects received peanut flour instead in another vehicle food of their choice. There were no significant differences in reaction type between the 362 children who ate the standard cookie and the 16 children who ate the peanut flour in another vehicle (x^2= 2.21, p=0.53).

ACCEPTED MANUSCRIPT

245 subjects showed no reaction to the cookie single dose OFC (Table 3). For 133 subjects, some comment indicative of a possible reaction was recorded in the written OFC records. Sixty-seven reported subjective symptoms only. Sixty-six events were considered objective, but 58 of these did not meet the predetermined criteria. The very mild and transient objective symptoms that did not meet the predetermined criteria included non-persistent usually single sneeze, non-persistent usually single cough, small areas of transient erythema, and fewer than 3 hives lasting <5 minutes. Eight participants experienced objective events that met the predetermined criteria (Table 4). All eight subjects who met the pre-determined criteria consumed the cookie not an alternative vehicle. No participant experienced more than a mild reaction; four of the 8 most objectively reacting subjects were treated with oral antihistamines. No other subject was treated and none received epinephrine.

Multivariable regression analysis showed no significant differences for age and centre, reaction type or participant/ non participant status. The eight subjects who met the predetermined objective criteria were no different in age to others included in the study (Table 4).

Study centre and reaction type were not significantly related to diagnostic entry criterion (x^2=3.39, p= 0.76). Subjects' sex was not significantly related to reaction type (x^2=4.76, p= 0.19).

Univariate analyses showed peanut spIgE, Ara h1, Ara h2, Ara h3, Ara h8, Ara h9 spIgE levels and total IgE levels had no effect on inclusion criterion met or participant/non-participant status, (p 0.21 - 0.99) (Table 5). Peanut SPT differed between study centre location (η_p^2 = 0.02, p = 0.03) with a small effect size (11), but not for reaction type (p=0.25). Irish subjects had the lowest mean wheal size (M= 9.50 mm, SD= 2.66) and Australia the highest (M=15mm, SD=6.47). No other skin or blood tests were significant for either type of reaction or location (p>0.05).

ACCEPTED MANUSCRIPT

Adherence to precautionary labelling at study entry was significantly lower in Australia where 76% ignore labelling compared to Ireland (33%) and US (36%) ($x^2 = 66.21$, $p<0.001$). Proxy and self-reported adherence to precautionary allergen labelling did not significantly change from T1-T2 and was unaffected by age of child, study centre or diagnostic criteria met (p= 0.82-0.42).

Food allergy-related quality of life

Baseline scores (before OFC) in the FAQL-PF predicted likelihood of reporting subjective vs objective symptoms (after OFC) (p=0.001). In effect, children who later experienced subjective symptoms to the single dose of peanut had the most adverse impact on FAQL at baseline (Mean =2.6, SD= 1.4). Those who did not experience any reaction had the best FAQL (lowest burden) at baseline (Mean= 1.8, SD=1.3). This provides further evidence of the association between clinical and psychological factors in food allergy.

There was a significant main effect for time from T1 to T2 for parent reported proxy FAQL-PF (η_p^2 =0.24, p=0.014), with a medium to large effect size (11), where parents reported an improvement in FAQL for their children from baseline to 1 month post protocol. There was a significant three-way interaction between age, sex and time (η_p^2 = 0.11, p=0.014) with a medium effect size (11). Regardless of age or sex of child, parents reported improved FAQL at T2. Younger boys experienced a higher impact, whereas as age increased, parents reported more adverse impact for girls. Diagnostic criteria and type of reaction elicited in the single dose study were not significant.

ACCEPTED MANUSCRIPT

Children's self-reported FAQL-CF also improved from baseline (T1) to 1 month post protocol (T2) (η_p^2=0.5, p=0.001) with a very large effect size (11). Again there was no effect on FAQL by inclusion criteria met or type of reaction (p=0.158).

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Discussion

The novel single-dose PATS findings strongly support the safety of the statistically determined ED_{05} based upon population dose-distribution modelling (2) for administration to a non-selected patient population. The protocol was very acceptable to families and was clinically very safe. This approach offers the opportunity to identify the most dose-sensitive peanut allergic population in a safe and efficient manner. It could be adapted for other major allergenic foods.

Population EDs can be estimated by statistical dose-distribution modelling of individual patient threshold doses (2-4). ED estimates can vary depending upon the choice of model. The single-dose PATS approach serves as a useful way to validate the ED estimates and select the best parametric model. In this single-dose PATS, the percentage of patients reacting with the predetermined objective criteria (2.1%) was lower than predicted from the log-normal model (5%; 95% CI of 3.1-7.8%). Several reasons could explain the observed difference between the predicted 5% versus the observed 2.1% rate. First, selection bias toward more highly sensitive patients could have occurred with the 750 peanut-allergic subjects in the modelled dataset as many of the patients included in the dataset were from tertiary allergy clinics which could contribute to a bias toward a more sensitive peanut-allergic population (2,3), though this study group of consecutive patients was also recruited in tertiary centres. Second, although objective responses were used in the clinics conducting threshold challenges and the PATS, the objective criteria used to establish the lowest observed adverse effect level (LOAEL) for some of the patients may not have been as stringent as the criteria established for the PATS. In particular and among the mild transient reactions that did not meet the predetermined objective criteria, 13 additional patients experienced hives (a single hive in 8 cases, 2 hives in 4 cases, and 3 hives in 1 case, all lasting less than the stipulated 5 minutes). Had these 13 cases been counted as positive response to the single-dose challenge, the reaction rate would have

ACCEPTED MANUSCRIPT

been 5.5%. Given these possibilities, the log-normal model used appears to be reasonable and appropriately conservative for use in the estimation of EDs for peanut.

Population modelling of individual threshold doses can be used to establish public health measures such as the control of precautionary allergen labelling (PAL). In Australia, a Reference Dose for peanut of 0.2 mg peanut protein was established from estimates of the ED_{01} (2). The ED_{01} was selected by the VITAL Scientific Expert Panel (VSEP) because it is predicted to protect 99% of the peanut-allergic population. However, based on the mild and transient responses encountered in PATS, the use of the ED_{05} as the basis for the peanut Reference Dose would be a more reasonable and implementable risk management decision.

PAL abounds in many marketplaces but stakeholders find fault with the approach because use of PAL bears little relationship to actual risk (13,14). Almost 50% of the study population were routinely ignoring precautionary labelling. PATS has validated the $ED_{05,}$ so the medical and food science communities, manufacturing industry, and public health authorities should consider adopting this model. This would assist in establishing an ED_{05}-based peanut Reference Dose to be used in quantitative risk assessment to underpin PAL, backed by sound scientific evidence, that protects the vast majority of the peanut allergic community.

No centre appeared to have a uniquely more sensitive study population than the other two, suggesting this protocol and the predetermined criteria used for assessing single dose OFC could be used in other centres. Ireland had far more challenge-proven cases than the other centres but lower average ages than the US centre, and Australian patients had larger peanut SPT and paid less attention to precautionary advisory labels. These inter-centre demographic and diagnostic differences did not influence the primary or secondary outcomes of the study.

ACCEPTED MANUSCRIPT

The predetermined approach to offer the study to all peanut allergic subjects in 3 distinct geographical regions, the comparison of characteristics of participants and non-participants, the permissive entry criteria and the pre-determined conservative case definition combine to address the most common criticism of OFC studies: How representative of the general peanut allergic population are the subjects who volunteered? This study showed peanut allergic children in each centre were broadly similar, that severe reactors were included and, critically, that participants appeared not to differ clinically from non-participants. While we did not prospectively record previous reaction severity, all subjects were recruited from referred populations seen for their peanut allergy in tertiary/national referral centres, so it is likely the representation of the severe end of the clinical spectrum of peanut allergy in this study population is at least similar than that reported peanut allergy norms.

Limitations of the study

Many of the patients recruited were diagnosed without the gold standard double-blind placebo controlled food challenge. However, the intended recruitment strategy was to recruit relatively unselected but near-certain cases, to capture the whole spectrum of cases, which is often not included in incremental dose challenge studies. Our data show no differences in demographic details or serological findings between participant and non-participants or between reactors and non-reactors or between the 8 most certain objective reactors and other groups. The inclusion and exclusion criteria appear to have been well constructed, based on established clinical methods used elsewhere, clinical history and SPT and spIgE levels above determined decision points (7).

Subjects did not undergo placebo challenges, just an active-dose cookie, given once. Placebo doses would have required doubling attendances to more than 700 visits and we considered the projected likelihood of significant reactivity of around 5% in the single dose study did not justify a placebo

ACCEPTED MANUSCRIPT

arm. It is notable that 65% of subjects reported no reaction at all to the ED_{05} cookie, despite knowing it was an "active" dose. Intentionally liberal documentation of reported symptoms and having a set of fixed, pre-test criteria for an objective reaction allowed *post hoc* distinction of subjective from objective reactors, though relatedness of any reaction to the single dose was difficult in real time due to the lack of options normally available in routine OFC, such as waiting longer between doses and repeating doses (1,7). Subjective reactors had lower pre-test FAQL than objective reactors and non-reactors which suggests anxiety may play a role in reports of mild/subjective reactions at low doses in the community and in DBPCFC (15) and also possibly in reactions to placebo doses during DBPCFC (16).

PATS was an assessment of low-dose sensitivity in a population of peanut allergic subjects at a single time point and further studies are needed to assess both population-level and individual subjects' variation in low-dose sensitivity over time. Standard, incremental DBPCFC does not correlate well with reported severity of community reactions (17) and dose is only one variable to be considered in the difficult assessment of severity of food allergy. (18)

The PATS study offers a new clinical paradigm and methodology with regards to assessing clinical risk; this current study may potentially define the 5% of patients who are most dose-sensitive. It confirms previous findings that validated questionnaires assessing FAQL show patients gain nearly as much from a "failed" OFC as they do from a "passed" OFC, probably due to decreased uncertainty about the next and future reactions. (13). This tangible impact could promote adoption of PATS single dose peanut challenges in units not currently performing diagnostic multi-dose OFC.

ACCEPTED MANUSCRIPT

The single dose protocol does not replace current clinical food challenges which are critical for definitive diagnosis of food allergy but would provide extra clinical information of patients' level of risk, related to dose, and could help inform consumer choices and physician advice to patients regarding PAL (14, 15); single dose challenges could be done before starting a progressive clinical food challenge to identify the most highly sensitive patients and reduce any risks associated with the use of higher doses used in clinical food challenges. PATS suggests clinical validation of other allergenic food sources could be addressed in similar studies, where the population dose-distribution has been modelled using sufficient threshold data. Clinicians may be able to use PATS single dose OFCs widely as they are easier to perform than routine diagnostic OFC or DBPCFC.

Conclusion

The novel single dose OFC, based upon the statistical dose-distribution analysis of past challenge trials, is a clinically safe and efficient approach to identify the most highly dose-sensitive population of food-allergic people and it improves food allergy related quality of life. The validation of the ED_{05} will also assist regulators, public health agencies and manufacturers in the establishment of approaches to allergen management that will protect the vast majority of food-allergic consumers/patients.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Author's contributions

JOBH is the guarantor of the study and wrote manuscript drafts.

JOBH, KA and SLT conceived the study initially.

WS and KA contributed to study design, clinical supervision and data analysis.

JN produced OFC materials, monitored the study and contributed to data analysis.

JB and ADG contributed to study design and data analysis.

GDG performed data analysis.

GZ and LG contributed to study design, selection of statistical methods and challenge performance.

All authors have contributed to manuscript drafts and have seen and approved the final version

Acknowledgement

This project is funded by Food Allergy Research & Resource Program (FARRP) and supported by Grant Number 1UL1TR001102-01. Dr. Eyal Oren (Northshore Allergy), Colette Hurley, and other nursing or medical staff who assisted recruitment and challenges.

Conflicts of Interest

JOBH, KJA, LCG, GZ, JN, GDG, ADG none to declare. WGS receives consulting fees from Sanofi USA (Cambridge, MA) and Epiva Biosciences (Cambridge, MA). SLT and JB receive research support from over 80 worldwide food companies through the Food Allergy Research & Resource Program consortium and from the U.S. Department of Agriculture. SLT and JB receive royalty payments from Neogen Corp. (Lansing MI, USA) related to the sales of food-specific immunoassay kits. SLT receives consulting fees from ConAgra Foods (Omaha NE, USA), Kellogg's (Battle Creek MI, USA), and Keller & Heckman (Washington DC, USA).

ACCEPTED MANUSCRIPT

References

1. Sampson HA, van Wijk RG, Bindslev-Jensen C, Sicherer SH, Teuber S, Burks AW, Dubois AEJ, Beyer K, Eigenmann PA, Spergel JM, Werfel T, Chinchilli VM. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology–European Academy of Allergy and Clinical Immunology PRACTALL consensus report J Allergy Clin Immunol 2012; 130 (6); 1260–1274. DOI: http://dx.doi.org/10.1016/j.jaci.2012.10.017

2. Taylor SL, Baumert JL, Kruizinga AG, Remington BC, Crevel RWR, Brooke-Taylor S, Allen KJ; Allergen Bureau of Australia & New Zealand, Houben G. Establishment of Reference Doses for residues of allergenic foods: report of the VITAL Expert Panel. Food Chem Toxicol. 2014;63:9-17. doi: 10.1016/j.fct.2013.10.032.

3. Taylor SL, Crevel RWR, Sheffield D, Kabourek J, Baumert J: Threshold dose for peanut: risk characterization based upon published results from challenges of peanut-allergic individuals. Food Chem Toxicol 2009, 47(6):1198–1204.

4. Allen KJ, Remington BC, Baumert JL, Crevel RWR, Houben GF, Brooke-Taylor S, Kruizinga AG, Taylor SL. Allergen reference doses for precautionary labeling (VITAL 2.0): clinical implications. J Allergy Clin Immunol. 2014;7:156–164. . doi: 10.1016/j.jaci.2013.06.042.

5. Zurzolo GA, Allen KJ, Taylor SL, Shreffler WG, Baumert JL, Tang MLK, et al. Peanut Allergen Threshold Study (PATS): validation of eliciting doses using a novel single-dose challenge protocol. Allergy, Asthma & Clinical Immunology. 2013;9(1):35.

6. Osborne NJ, Koplin JJ, Martin PE, Gurrin LC, Thiele L, Tang ML, et al: The HealthNuts population-based study of paediatric food allergy: validity, safety and acceptability. Clinical & Experimental Allergy 2010, 40(10):1516–1522.

7. NIAID-Sponsored Expert Panel. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, et al. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. J Allergy Clin Immunol. 2010;126:S1–S58. doi: 10.1016/j.jaci.2010.10.007

8. DunnGalvin A, Cullinane C, Daly DA, Flokstra-de Blok BM, Dubois AEJ, Hourihane JO.Longitudinal validity and responsiveness of the Food Allergy Quality of Life Questionnaire - Parent Form in children 0-12 years following positive and negative food challenges. Clin Exp Allergy. 2010;40(3):476-85. doi: 10.1111/j.1365-2222.2010.03454.x

9. Sampson HA, Munoz- Furlong A, Campbell RL et al Second symposium on the definition and management of anaphylaxis: Summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium., J Allergy Clin Immunol 2006;117(2): 391–397. DOI: http://dx.doi.org/10.1016/j.jaci.2005.12.1303

ACCEPTED MANUSCRIPT

10. Sullivan, G.M. and Feinn, R., 2012. Using effect size-or why the P value is not enough. Journal of graduate medical education, 4(3), pp.279-282.

11. Field, A., 2013. Discovering statistics using IBM SPSS statistics. Sage.

12. Tabachnick, B.G. and Fidell, L.S., 2001. Using multivariate analysis. California State University Northridge: Harper Collins College Publishers.

13. Zurzolo GA, Koplin JJ, Mathai ML, Tang MKL, Allen KJ: Perceptions of precautionary labelling among parents of children with food allergy and anaphylaxis. Med J Aust 2013, 198(11):621–623.

14. DunnGalvin A Chan CH, Crevel R. et al Precautionary allergen labelling: perspectives from key stakeholder groups. Allergy. 2015;70(9):1039-51. doi: 10.1111/all.12614.

15. Ballmer-Weber BK, Fernandez Rivas M, Beyer K et al. How much is too much? Threshold dose distributions for 5 food allergens J Allergy Clin Immunol 2015;135(4), 964–971 DOI: http://dx.doi.org/10.1016/j.jaci.2014.10.047

16. Ahrens B, Niggemann B, Wahn U, Beyer K. Positive reactions to placebo in children undergoing double-blind, placebo-controlled food challenge. Clin Exp Allergy. 2014;44(4):572-8. doi: 10.1111/cea.12284.

17. Hourihane JO'B, Grimshaw KEC, Lewis SA et al. Does severity of low-dose, double-blind, placebo-controlled food challenges reflect severity of allergic reactions to peanut in the community? Clinical and Experimental Allergy 2005 Sep;35(9):1227-33.

18. Turner PJ, Baumert JL, Beyer K et al Can we identify patients at risk of life-threatening allergic reactions to food? Allergy. 2016 Sep;71(9):1241-55. doi: 10.1111/all.12924.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Legends

Figure Flow diagram of subject recruitment and participation

Table 1. Inclusion and Exclusion Criteria

Table 2. Demographic comparison of participants to non-participants

Table 3. Primary Outcomes (reaction to single dose food challenge) per centre.

Table 4. Participants who met the predetermined objective reactivity criteria/case definition

Table 5. Reaction type vs. Mean values for Skin and Blood Tests

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Age between one to eighteen years old inclusively.	Medically unfit for challenge according to local unit OFC guidelines/protocol (e.g. high fever, unwell with intercurrent illness)
Evidence of peanut allergy by one of the following;	Oral corticosteroids within 14 days prior to challenge
History of unequivocal exposure (including accidental) and typical acute allergic reaction within the preceding 2 years and positive peanut SPT (performed according to local clinical protocols) /specific IgE.	Episode of anaphylaxis of any cause in the 4 weeks prior to challenge
Positive oral food challenge with peanut performed within 2 years - either open oral food challenge or DBPCFC (Double-blind, placebo-controlled food challenge)	Use of antihistamines within 5 days of oral food challenge
Peanut never ingested, but sensitisation to peanut above the 95% positive predictive value (PPV) for clinical allergy, i.e. peanut serum IgE $\geq$ to 15kU/L (by CAP FEIA) and/or peanut SPT wheal size $\geq$ to 8mm (7) within 2 months of the single dose challenge.	Asthma that is not well controlled as demonstrated by FEV_1 <85% of predicted best.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 2. Demographic comparison of participants to non-participants

	Participants			Non-Participants		
	Cork	**Melbourne**	**Boston**	**Cork**	**Melbourne**	**Boston**
Initial Number	124	126	128	63	24	53
Sex (%Male)	61%	56.3%	55.5%	60.3%	70.8%	71.7%
Age (Mean yrs)	6.36	7.63	6.55	6.78	8.54	6.65
Final Number*	124	126	128	63	24	35
Inclusion criterion met:**						
Typical reaction<2years	68	60	74	38	12	19
Positive OFC<2years	43	16	2	8	1	2
SPT/spIgE > 95% PPVs	13	50	52	17	11	14

*18 participants in Boston did not wish to participate immediately after initial recruitment and therefore no diagnostic information was gathered.

** Many subjects met both entry criteria 1 and 2 but only the single one entered in the restricted data file option is reported here.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 3 Primary Outcome (reaction to single dose food challenge) per centre

	Total	Cork	Melbourne	Boston
		Participants		
Active Eligible Participants (completed OFC)	378	124	126	128
		Outcome Group		
Total	378	124	126	128
Non-reactors	245	94	65	86
Reactors	133	30	61	42
Subjective Reactors	67	19	30	18
Objective Reactors				
Total Objective	66	11	31	24
Meeting predetermined criteria	8	1	3	4

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

91 **Table 4. Details of 8 subjects who met the predetermined objective reactivity criteria/case definition**

Participant Number	Location	Age (yrs)	Sex	Diagnostic method	Peanut Wheal (mm)	Peanut SpIgE kUA/L	SpIgE rArah1	SpIgE Arah2	Outcome
35	Ireland	11	Female	History of typical exposure & reaction & positive SPT/ spIgE	15	69.10	11.20	59.20	Rhinoconjunctivitis
40	Australia	15	Male	History of typical exposure & reaction & positive SPT/ spIgE	13	2.06	0.53	1.74	Urticaria
43	Australia	9	Male	History of typical exposure & reaction & positive SPT/ spIgE	18	N/A	N/A	N/A	Vomiting
95	Australia	2	Female	Peanut never ingested but positive SPT/spIgE> 95% PPVs	13	N/A	N/A	N/A	Vomiting
31	U.S.	9	Male	Peanut never ingested but positive SPT/spIgE> 95% PPVs	11	0.36	0.10	0.14	Urticaria
97	U.S.	2	Male	History of typical exposure & reaction & positive SPT/ spIgE	N/A	100.00	14.80	100.00	Urticaria
109	U.S.	1	Male	History of typical exposure &reaction & positive SPT/ spIgE	N/A	57.70	0.10	49.60	Urticaria
124	U.S.	4	Male	History of typical exposure & reaction and positive SPT/ spIgE	N/A	46.70	14.70	16.20	Rhinorrhoea

ACCEPTED MANUSCRIPT

92 **Table 5. Reaction type vs. Mean values for Skin and Blood Tests**

	Total IgE	Peanut spIgE	Peanut SPT Wheal (mm)	rAra h1 spIgE	rAra h2 spIgE	rAra h3 spIgE	rAra h8 spIgE	rAra h9 spIgE
Type of reaction (n)								
Non-reactor (245)	490.46	28.18	11.69	11.11	22.52	4.88	1.49	0.74
Subjective (67)	1164.89	46.07	15.23	23.42	32.86	9.33	0.74	0.11
Objective (66)	1130.80	39.46	13.60	14.87	31.90	3.13	1.21	0.19
Satisfies pre-determined criteria (8)	290.67	45.99	14.00	8.18	45.03	2.35	0.13	0.31

93

94

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Figure X. Flow of participants through study

