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Single dose challenges in the diagnosis and management of cow's milk allergy in infants.

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Submitted as part of MSc by Research to National University of Ireland, Cork.

Research conducted through the Department of Paediatrics and Child Health, University College Cork.

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Declaration

I, Yvonne d'Art (student ID 117224095), declare that this thesis is my own original work and has not been submitted for another degree at University College Cork or elsewhere.

ABSTRACT

Background:

Cows milk protein allergy (CMPA) is one of the most common food allergies in infancy. While it usually resolves slowly over time in most cases, it significantly disrupts family life and compromises affected childrens' nutrition and growth. Parents often display significant anxiety about this condition and we speculated if this anxiety predates or develops in response to the onset of CMPA in their child. Single dose challenges are a new method of assessing dose reactivity in food allergic children. We recruited children referred for evaluation of CMPA to a randomised, controlled trial of single dose exposure to cows milk, using the validated dose of milk that would elicit reactions in 5% of CMPA subjects - the ED_{05}, before implementation of graded exposure to CM (using the 12 step IMAP Milk Tolerance Induction Ladder) at home.

Methods:

60 infants were recruited from referrals to 2 tertiary allergy centres and 1 secondary level allergy clinic. Inclusion criteria were age <12 months, a convincing CM allergic reaction <2 months before assessment and positive skin prick test (SPT) to milk +/- raised SpIgE to milk. Children were randomised 2:1 to a single dose of the ED_{05} for CM - (0.5mg milk protein) given as liquid CM (0.015mls) and observed for 2 hours post ingestion - or to no dose.

Results:

60 patients were recruited, 57 (95%) were followed to 6 months, 3 intervention subjects were lost to follow up. By 6 months 27/37 (73%) intervention subjects had reached step 6 or above on the milk ladder compared to 10/20 (50%) control subjects (p=0.048). By 6 months 11/37 (30%) intervention subjects reached step 12 (ie drinking unheated cow's milk) compared to 2/20 (10%) of the controls (p=0.049). 12 months post randomisation 31/36(86%) of the intervention group and 15/19(79%) of the control group were on step 6 or above.However 23/36(64%) of the intervention group were at step 12 compared to 7/19(37%) of the control group. Maternal state and trait anxiety were significantly associated with their infants' response/progress on the milk ladder and with changes in skin prick test and spIgE levels at 6 and 12 months.

Conclusion

Using the 12 step IMAP milk ladder accelerates natural tolerance induction in infants under 12 months with CMPA. A supervised single dose at the ED_{05} significantly accelerates this further, probably by giving parents the confidence to proceed. Maternal anxiety generally reflects infants' progress towards tolerance but preexisting high levels of maternal anxiety are associated with poorer progress.

Acknowledgements

Firstly I would like to thank my supervisors, Professor Jonathan Hourihane (my husband), Dr Aideen Byrne and Dr Louise Gibson for their time, guidance, encouragement and sharing of their expertise over the last three years.

I would like to thank everyone at INFANT in Cork especially Marc O'Sullivan who was always available to help with my laboratory work.

Similarly I would like to thank all of the staff in CHI Crumlin both research and clinical who were always there to help on my many visits but especially Alicia Wallace whose organisational skills are second to none.

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Finally a massive thank you to my family- my husband, Jonathan, and children Aisling, Claire, Joe and Tom- for their support and encouragement and for putting up with my many absences so that I could complete this study. Thank you to my wonderful mother, Etta, for the many meals and nights of accommodation provided on my trips to Dublin and finally to my late father, John, without whom I would never have entered the world of medicine.

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Abbreviations

CMPA	Cows milk protein allergy
CUH	Cork University Hospital
IMAP	International Milk Allergy in Primary Care Guideline
OFC	Oral food challenge
OIT	Oral immunotherapy
SAE	Serious adverse event
SOP	Standard operating procedure
SpIgE	specific IgE
SPT	Skin prick test

INTRODUCTION

Food allergy affects 4% of Irish infants, with the dominant foods being cow's milk, egg and peanut. Cow's milk protein allergy (CMPA) is one of the most common food allergies in infancy and childhood, affecting approximately 1% of Irish infants (1) which can have major and lasting impacts on the affected child's physical and mental health and also on the health of the whole family.

The impact of food allergy on children and their families is substantial, involving safe dietary substitution of growth-critical foods, food safety awareness and the availability and confidence in use of rescue medications, including adrenaline injections.

At present in Ireland the vast majority of children with food allergies need to be referred to secondary care for definitive diagnosis and treatment. Due to the limited availability of these services children and their families may have to wait for months before being seen in established allergy clinics in Dublin or Cork or in 15 local units, only 2 of which (Galway and Portlaoise) have any particular resources for allergy care, such as skin testing or food challenge. Families may push for allergy "testing" in the private sector or just have blood tests taken by their family doctor, both of which can lead to over diagnosis and unwarranted dietary exclusions and even adrernaline prescriptions, without training or support.

Children with food allergies and their families have worse quality of life (QOL) than children with severe rheumatological disorders and diabetes (2, 3), with a major stressor being the levels of uncertainty about future reactions and the remote - but not zero - risk of fatality (4). Appropriate access to diagnostic and support services decreases these risks and completion of formal oral food challenge (OFC - the only definitive procedure), improves Food Allergy related Quality Of Life (FAQL) substantially, even in children who do not pass a formal oral food challenge (OFC) and are shown to be allergic (5, 6). The major reasons for this improvement are removal of uncertainty and an increased feeling of safety and competence in self-care. Access to expert medical support to complete diagnostic OFC is difficult in Ireland as this resource is only comprehensively available in 4 sites in Ireland (CHI - Crumlin, Tallaght and Connolly, and CUH, Cork) and to a limited extent in Galway and Portlaoise. This has led to long waiting lists and some children may have to wait for years to have a diagnostic OFC.

Traditionally thought to be a transient allergy, with a high rate of resolution by the age of school entry, the natural history of cow's milk allergy (CMA) in populations referred to specialist centres appears to be much worse. An early study in the early 90s (7) which included 100 children, reported that 78% of CMP allergic children were tolerating milk by age 6. However more recent studies have found a much lower rate of resolution. In 2007 Skripak el al enrolled 807 cow's milk allergic children (median age at enrolment 13 months) into a study looking at tolerance acquisition. In this study only 6% were tolerant of unheated milk by 2 years of age and 19% by 4 years (8). The authors accept that this relatively poor prognosis may be a result of their highly atopic, tertiary referral population. However their rates of asthma, eczema and allergic rhinitis were similar to those found in previous studies, leading them to speculate that it may

also be that the nature of CMPA has changed over time and it may now be truly a more persistant disease.

A further study by Kim et al in 2011 also showed a worse than previously thought rate of resolution. Their results showed that even in children who were baked milk tolerant at diagnosis only 14% were tolerant to unheated milk 12 months after enrolment with only 65% tolerant of unheated milk products after 5 years of follow up (9).

Current diagnosis and treatment of cow's milk allergy

Diagnosis of cow's milk allergy is made on the history of symptoms of a typical IgE mediated reaction (immediate onset of symptoms such as rash, urticaria, facial oedema, vomiting, diarrhoea, wheeze, anaphylaxis which resolve within hours) supported by diagnostic testing using skin prick tests +/- spIgE and in some cases OFC.

Historically, treatment of CMPA was avoidance of cow's milk protein until natural acquisition of tolerance occurred, typically at around school going age (10). This therefore involved prolonged avoidance of dairy products for years and adequate nutritional substitution. This in itself could be problematic and expensive for families in Ireland, with limited access to dietetic expertise in some areas.

Why then do we need to push infants to acquire tolerance to cow's milk protein more quickly than would naturally occur?

Among the many reasons are growth and bone health. It has been shown that infants with CMPA are smaller, lighter and have a lower bone density aged 5-15y than those who do not (11). Earlier resolution may offer the advantages of an earlier unrestricted

diet reducing possible food avoidance-related nutritional risks.Dietary and taste preferences are established in the 1st year of life, so it is important that babies are introduced to a wide variety of foods as soon as possible.

Early resolution of CMPA may alleviate the anxiety within families which not only affects the allergic child but also their parents and siblings. It helps with safe social integration outside the home which is especially important as most children will attend childminders/creches/playschool at a young age before actually starting primary school. This can improve the family's quality of life by reducing anxieties around the risk of reactions related to accidental exposures.

Ability to consume normal milk and dairy products avoids unvalidated 'allergy' tests and unnecessary and at times, dangerous exclusion diets. For example, many parents will try goat's milk to which the CMPA child will also most likely react. They will often also use plant based milks which are not nutritionally complete for children (10).

It has been found that tolerance acquisition can be accelerated by the introduction and incremental exposure to baked milk products, thought to be due to gradually increased exposure to milk proteins, whose allergenicity is altered by the cooking process (10).

In the study by Kim et al, baked milk was introduced into the diet of children who were baked milk tolerant at diagnosis(9). 14% were unheated milk tolerant after 12 months and 76% after 60 months. In their comparison group which received normal care (ie avoidance of milk) only 7% were unheated milk tolerant after 12 months and 33% after 60 months of follow up.

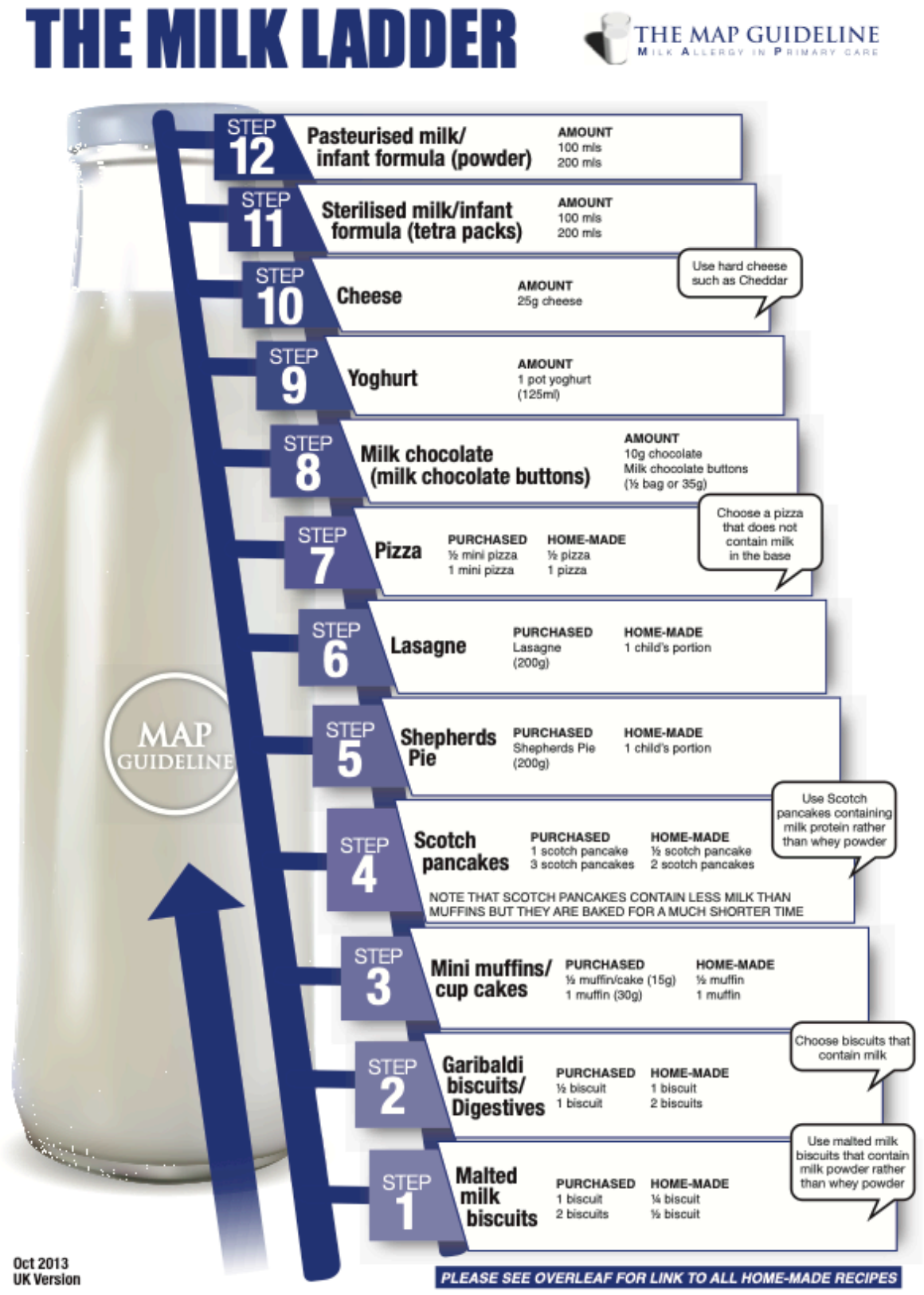

Figure 1 IMAP 12 step milk ladder

In Ireland the iMAP milk ladder is used to facilitate the introduction of baked milk(12). Originally devised to assist with tolerance acquisition in non- IgE mediated CMPA, it is now also increasingly used in IgE mediated allergy as shown by a recent survey.(13)

Using this ladder the child starts at the bottom rung consuming well-baked milk products (Step1) and progresses at intervals (decided by the healthcare professional caring for the child) to consume less well cooked milk until he can safely consume unheated milk (Step 12) without any reaction. A six step milk ladder was launched mid way through this study's recruitment period and the study group agreed to continuing with the 12 step ladder to maintain study integrity.

The milk ladder is also used in the UK and Australia for the treatment of CMPA. However current BSACI guidelines advise reintroduction of milk from 12 months of age only(12.) Home introduction is advised only if initially there were mild symptoms on noteworthy exposure, no reaction in the last 6 months and a significant reduction in SPT size. In Australia current guidelines advise avoidance of milk until 2 years of age when an in-hospital baked milk challenge is offered. If baked milk is tolerated they are then referred for a whole milk challenge before home introduction. In the best case scenario here the child is at least 2 years and often 3-5 years before the whole milk challenge is done leading to extensive periods of avoidance (personal communication Vicki McWilliam, Dietitian, RCH, Melbourne, Australia).

Current practice in the tertiary allergy units in Cork and Dublin is to start using the milk ladder at diagnosis, irrespective of age, SPT, spIgE levels or severity of initial reaction and once the child is weaned onto solid foods and therefore able to consume the relevant foods on the ladder.

Immunotherapy for food allergies by oral and non-oral routes is being studied intensively at present but is unlikely to reach the clinic soon apart from peanut immunotherapy (15). Studies into oral and epicutaneous peanut immunotherapy are ongoing. One commercial product for oral peanut immunotherapy has been licenced in the US and Europe but is not yet available in Ireland. Milk OIT is widely used in Southern Europe, however it is not often used in Ireland. Berti et al(16) showed that OIT with milk is safe and effective even in infants. This study included 68 infants who had home OIT. 97% reached the target of the protocol (tolerating 150mls of milk) and taking a median time of 5.5 months. However the study included 27 infants who had a negative OFC at baseline and there was no control group. The main disadvantage of their OIT protocol is that it is necessary to do the updosing to the next dose of milk in a clinical setting until reaching a dose of 40mls which entailed hospital visits every 3 weeks. In comparison in Ireland the iMAP milk ladder is initiated at home and patients can progress without the need for multiple hospital visits.

The new role of single dose food challenges

The single dose OFC, based upon the statistical dose-distribution analysis of past challenge trials, is an efficient approach to identify the most highly dose-sensitive patients within any given food-allergic population. Standard oral food challenge (OFC) protocols use graded, incremental doses administered at short, fixed time intervals. It is not always possible to determine whether a reaction has occurred to a *discrete* threshold dose of allergen or alternatively has been the result of the *cumulative* dose consumed by the allergic individual at the time of reaction. The use of statistical dose-distribution modelling based upon the results of low-dose clinical challenges of peanut-

allergic individuals has been viewed as a strong approach to estimation of the population threshold for peanut. For example, 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 and this dose has been validated in a multicentre study, the Peanut Allergen Threshold Study (PATS) led by UCC, involving 375 peanut allergic children in Ireland, Australia and the US (17). We have also validated the ED_{05} for hazel nut (ED_{05}=1.5mg hazelnut protein, n=93) and milk (ED_{05}= 0.5mg milk protein, n=55) in EU-FP7 Project number 312147: IFAAM Integrated Approaches to Food Allergen and Allergy Risk. The data from a recently published paper involving 172 children , including 40 single-dose treated infants from this study, also support an estimated ED_{05} for cow's milk of 0.5mg of cow's milk protein (0.015mls of fresh cow's milk (18). This multicentre study incorporated children with CMPA from 4 centres (St Mary's Hospital, UK (Imperial), Hospital Clinico San Carlos and Hospital Universitario Infantil Niño Jesús, Madrid and Cork University Hospital, Ireland). The age range was 0-17 years. Twelve (7.0%, 95% CI 3.7%-11.9%) children experienced objective symptoms that met the predetermined criteria. One had mild anaphylaxis that responded to a single dose of adrenaline but the other 171 children experienced only mild symptoms, with no treatment needed. The centres had robust protocols for enrolment and used predetermined objective challenge-positive criteria to demonstrate true clinical reactivity.

In the original IFAAM study to validate the ED_{05} for milk (not published), screening and initial intervention with the single dose OFC with 0.5mg milk protein (0.015mls fresh full fat milk) took place on the same day if SPT was positive (>3mm in presence of at least a 3mm response to the positive histamine control). If SPT was negative the child was not eligible for the study, unless the clinical history was strongly suggestive and

there was an existing milk specific IgE >0.7KU_A/L from less than 2 months previously. If the reaction was more than 2 months previously the child went on to have a fresh milk challenge to confirm CMPA. An isolated positive spIgE blood test was not used as the sole entry criterion as many Irish babies with eczema/atopic dermatitis are unnecessarily tested in other settings for food-related spIgE, in the absence of the above suggestive clinical history. We used similar enrolment criteria in this study and the same objective challenge-positive criteria as these have been proven valid by the previous study (18).

The single-dose OFC is an open, not double-blind or placebo-controlled challenge, due to the young age of recruited children. Infants having routine allergy care in Ireland do not routinely have an OFC before being advised to start introducing baked milk, in the form of the milk ladder.

In this study they were not offered a placebo OFC because

i) only around 5% of tested children will be expected to react objectively to this dose,
ii) a single low dose is immunologically unlikely to initiate tolerance on its own, so a placebo arm is not necessary and
iii) the control group are actively receiving routine care.

In fact the contrary – sensitisation after a single low dose OFC - would probably be more likely in a naïve population, but this is not a naïve population, as they are by definition already allergic to cow's milk. Using the ED_{05} of milk, we would expect to find around 5% of tested children who react at this dose. In IFAAM, with a group of 55 there were 4 reactors = 7.5%, within the pre-study 95% confidence intervals of 0.2-

18% (19). These 4 children started the tolerance ladder in the same fashion as the non-reactors and did not experience additional problems.

There are no “stop criteria” in a single dose challenge unlike in routine OFC (18). Only objective criteria are used in the evaluation of single dose OFC reactions since that dose was calculated on the basis of challenge-associated objective responses (17,20).

During the previous single dose studies of peanut and milk ED_{05}s (PATS and IFAAM) it was evident to the investigators, but was not formally studied under the existing protocols, that recruited families got significant support and increased confidence from the evidence-based, expertly supervised demonstration of their child’s safety with a defined low dose of their problem food. In PATS all groups studied showed improved FAQL, even the small number of children (8/375) who reacted to the peanut ED_{05}.

In the IFAAM Cork group of 26 children with CMPA there was no formal follow up as part of the study protocol. Patients reverted to routine clinical care. However in my time in the allergy clinic, the team and I observed that, when reviewed in routine outpatient allergy clinic after the study, the children who had received the single dose challenge seemed to be achieving tolerance relatively quickly compared to the normal expected tolerance acquisition. I then contacted 12 patients who were 6 months post single dose and 5 who were 12 months post single dose. 7/12 (58%) children followed up to 6 months post single dose had reached the halfway point on the 12-step milk tolerance ladder and 3/5 (66%) followed for 12 months were fully tolerant of unheated milk. This rate of acquisition of tolerance is far in excess of that reported to date (8,9). It must be noted however that the age of the children in the Mount Sinai study (9) was

older than those in CUH, but this may be offset by the fact that the Mount Sinai children were known to already be baked milk tolerant at recruitment.

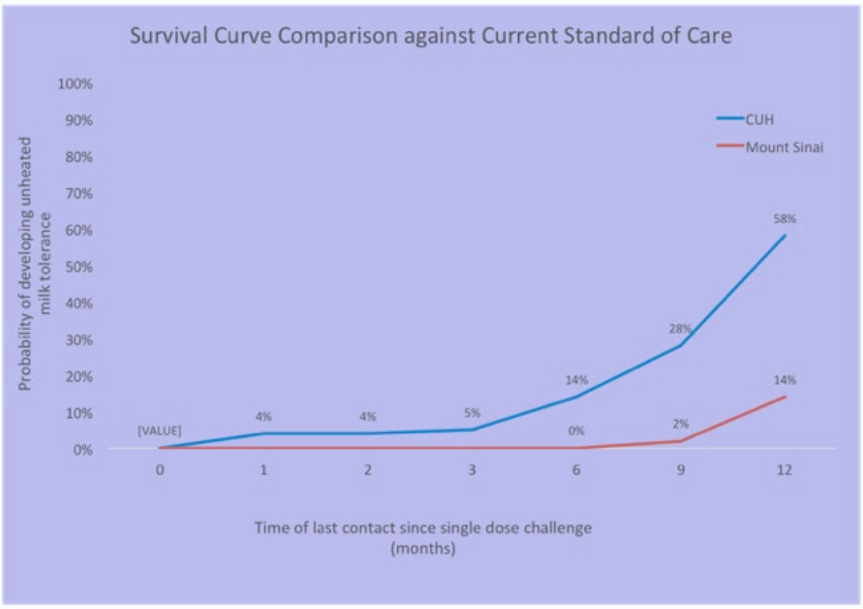

Figure 2 Comparison of survival curves from Mt Sinai New York (Ref 9) and Cork's unpublished IFAAM study data.

Our suspicion was that this acceleration of natural tolerance is due to increased parental empowerment and confidence in implementing the now standard advice about graded exposure to heated and then unheated milk. Parents have observed their child being exposed to a fixed low dose of milk protein and this may improve their confidence in implementing the milk ladder at home. It is also possible that starting at such a low dose as the ED_{05} allows exposure to heated milk to start at what is known in toxicology as the No Adverse Effect Level (NOAEL) (21) and that tolerance is facilitated by this low level initiation.

As there was no formal follow up as part of the IFAAM study we felt it was important to confirm our observations in a controlled trial. I applied to the National Children's Research Council for funding to do this and in 2017 received a Clinical Research Fellow scholarship to carry out this further research.

There has long been an impression among staff in the allergy services that they are dealing with a more anxious group of families than in the general population and also in a hospital population. It was thought that this high anxiety level was due to the disease itself and its impact on the family. However, in our more recent dealings with these families we formed an impression that the anxiety may pre-date the diagnosis of allergy and is subsequently worsened by the diagnosis. This leads us to consider the possibility that more anxious parents have children with allergies and a possible causal relationship. Some studies have suggested that there is a possible association between development of food allergy and antenatal anxiety levels. Alviani et al showed a possible association between the rates of antenatal stressful events, in particular, rates of maternally reported antenatal illness (22). However this was a small retrospective study with 32 mothers of food allergic children under 6 years of age which could have led to recall bias. Also 35% of their control group of 40 children without food allergy were atopic. Ideally the control group should have been non-atopic. In a Finnish study (23) it was found that the mother's chronic and moderate psychological distress (especially depressive symptoms) during gestation were associated with subsequent infant food allergy. They and others (reviewed in 24) suggest that chronic psychological distress exposure may be relevant for the programming of the foetal/infant immune system towards atopic diseases.

In our study we decided to take the opportunity while recruiting very young food allergic infants to look at this issue in more detail using validated State and Trait anxiety questionnaires (STAI) (25). The STAI has 2 parts. One part measures the level of anxiety at a point in time- the STAI-S or state anxiety. State anxiety is the current level of anxiety which reflects current circumstances and can change rapidly depending on external stressors. The second part measures overall anxiety-the STAI-T or trait

anxiety. Trait anxiety is the background anxiety which reflects ‘personality’ anxiety and is expected to remain constant over time.People with high trait anxiety show state anxiety increases more frequently than those with low trait anxiety because they tend to interpret a wider range of situations as dangerous or threatening. The individual’s perception of threat may have a greater impact on the level of state anxiety than the real danger associated with the situation (25). Maternal state anxiety would be expected to be high at diagnosis but decrease over time as tolerance to milk is achieved. We were interested in assessing if the trait anxiety level is higher than the norm in the mothers of milk allergic infants and if this affects the child’s longer term outcome and rate of acquisition of tolerance to cow’s milk.

Quality of life measures can be “generic” - about overall quality of life - or “disease-specific” -just relating to a particular condition. Food allergy-related Quality of life (FAQOL) measures were developed by our group and have been extensively validated and adopted to review both clinical outcomes in routine care (5) and in research studies, including OIT trials (26).

In this randomised, blinded study we wanted to formally measure the effect of these single dose challenges with the ED_{05} of milk (0.5mg milk protein or 0.015mls milk) on the acquisition of tolerance to milk in CMP allergic infants when compared to the impact of routine care, started at the same time point but without the single dose challenge.

We also wanted to investigate the levels of anxiety among mothers of these infants and the impact this has on the progression of their child’s disease.

METHODS

Research hypothesis

The null hypothesis is that no difference exists in rate of acquisition of tolerance to cow's milk between those who safely consume the ED_{05} for milk (0.5mg milk protein) at outset compared to those who receive the same level of care but not the single dose of milk ED05.

Ethical approval

Ethical approval was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Approval was granted on 26th September 2017. Recruitment began in December 2017 and the study finished in October 2020.

Study settings and management

In this study we recruited 60 cow's milk allergic children in the 2 fully established allergy services in Ireland in Cork and Dublin and also in another centre in Drogheda, where allergy care was offered by a General Paediatrician with a special interest in Allergy. Recruitment was from referrals received from primary care to 1 of the 3 centres: Cork University Hospital (CUH) Cork, Our Lady's Hospital for Sick Children Crumlin, Dublin, (OLCHC, including cases referred to Dr Byrne in Tallaght Hospital) and Our Lady of Lourdes Hospital, Drogheda. CUH and OLCHC are the only centres offering integrated allergy care including routine food challenges for any child referred. Drogheda offers more limited care up to and including low risk OFC. Cork is the local, secondary and tertiary referral centre for Munster and acts as the national centre for the South of Ireland and accepts tertiary referrals from elsewhere. OLCHC is Ireland's largest stand-alone paediatric hospital offering local, secondary and tertiary allergy

services to the Dublin region and the rest of Ireland. Possible candidates for the study who were referred to Dr Byrne's clinic in Tallaght, Dublin were offered appointments in OLCHC. The nature and culture of Irish healthcare provision means these centres already see most children with suspected IgE mediated cow's milk allergy. CUH and OLCHC have dedicated clinical research facilities.

The study was managed under the governance structures of the INFANT Centre (www.infantcentre.ie) UCC.

Blood immunological analysis was done by Prof Ronald van Ree in AMC, Netherlands.

Study design

After written informed consent was obtained children were randomised (by random number generation) to intervention (single dose oral food challenge with 0.5mg milk protein, followed by IMAP milk ladder implementation at home) or routine care (no challenge before starting the milk ladder) in a ratio of 2:1.

Infants were diagnosed with IgE mediated CMPA in the routine clinical way using a history of recent (within the previous 2 months) typical reaction to milk or milk products and a positive skin prick test (SPT) to milk. In the previous IFAAM study any patient whose reaction had been more than 2 months previously went on to have a full OFC to milk to ensure that they were still milk allergic. This validated the criteria for inclusion only if the reaction had been in the previous 2 months.

The single dose was prepared using the devised SOP (Appendix 1).

Before administering the single dose OFC study staff ensured the anaphylaxis kit was ready: Adrenaline for IM injection, Salbutamol by inhaler (+ spacer) or by nebuliser,

antihistamines and corticosteroids for oral or parenteral administration, and cannulae and fluids for IV infusion.

I supervised all the single dose oral food challenges. I am skilled and experienced in the assessment, diagnosis and emergency management of anaphylaxis. I performed a pre-challenge physical examination specifically paying attention to oral cavity, pre-existing skin lesions and chest auscultation. After full clinical assessment I administered the single dose of milk, using a syringe, to avoid any topical contact on the face or lips of the infant.

Blood pressure, pulse rate, oxygen saturations were measured before and at 15 minute intervals for 2 hours post single dose OFC.

Criteria for positive oral food challenge were any objective signs occurring within 2 hours of ingestion of the ED_{05}.

Objective signs include:

- 3 or more concurrent non-contact urticaria persisting for at least 5 minutes.
- Perioral or periorbital angioedema.
- Vomiting (excluding gag reflex).
- Evidence of circulatory or respiratory compromise (anaphylaxis eg, rhinoconjunctivitis, persistent cough, wheeze, change in voice, stridor, difficulty breathing and collapse).

Blood was taken at recruitment for immunological markers. It was spun and serum stored at -80C in UCCs Children's Biobank, until batched and transported to AMC,

Netherlands for analysis of spIgE levels to milk using Unicap technology (Thermofisher, Uppsala, Sweden).

Before administering the single dose food challenge I administered the validated Europrevall Food Allergy related Quality of Life (FAQL) questionnaires, using the FAQL-Parent Form to the mother. State and Trait Inventory (STAI) questionnaires were also completed. Mothers were told the questionnaires would not be read but would be immediately placed in a sealed envelope to be transferred to the psychologist. This is standard practice in psychological research, so that the mother can be reassured about the confidentiality of her answers.

Parents were instructed in the use of the iMAP milk ladder and requested to start the next day.

Skin Prick Test (SPT) was repeated at 6 and 12 months post randomisation, bloods were repeated at 6 months post randomisation and questionnaires completed at 1,3,6 and 12 months post randomisation.

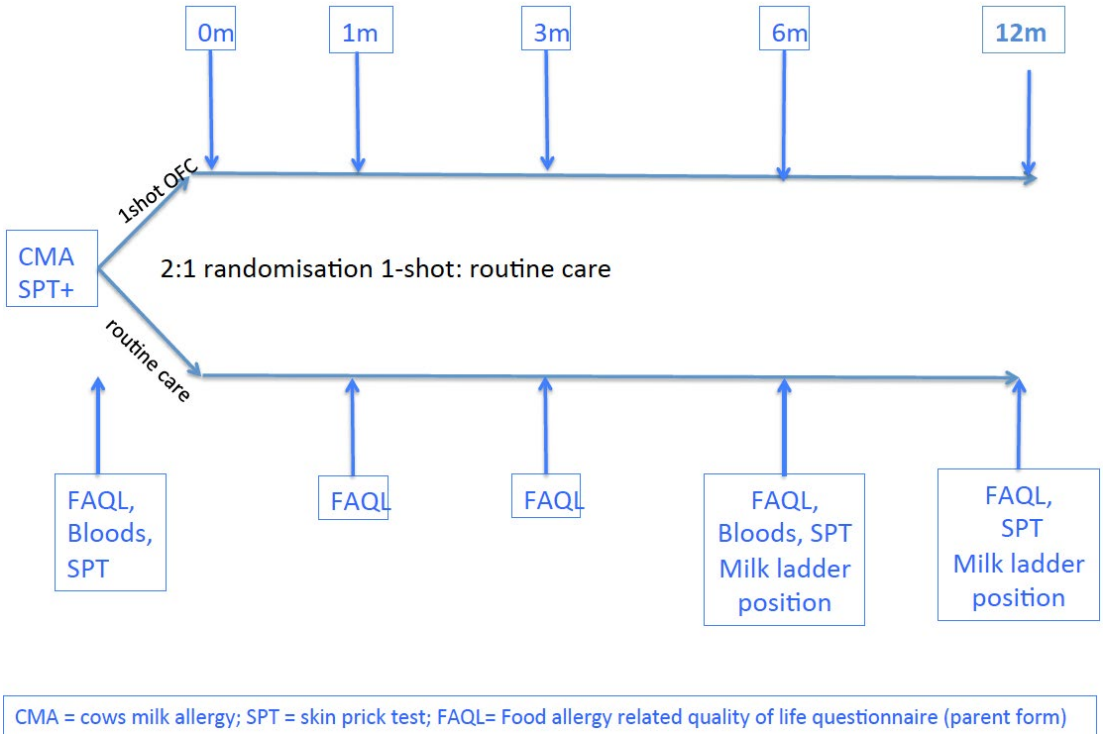

Figure 3. Study outline

Follow up was for 1 year from the date of challenge. At this time if further follow up was needed the patients reverted to normal allergy clinic care in CUH Cork or CHI-Crumlin.

Inclusion and exclusion criteria

Inclusion criteria

To be enrolled in this study each infant had to be in good general health (see below), be aged 12 months or less, and satisfy the following 2 study-specific major inclusion criteria:

1. Demonstrate strong clinical evidence of cow's milk allergy as defined by a typical unequivocal exposure and typical acute allergic reaction within the preceding 2 months

2. Have a positive allergen-specific SPT or sIgE to milk within 2 months of randomisation

Exclusion criteria

Study specific exclusion criteria were:

- The child was already tolerating baked milk products in its diet.
- The family was unable to give written informed consent in English.
- The child was considered medically unfit for challenge according to local unit OFC guidelines/ protocol (e.g., high fever, wheeze, unwell with intercurrent illness, antibiotics in previous 14 days).
- The child had received systemically administered corticosteroids within 14 days prior to challenge or used 1st generation antihistamines in previous 7 days or 2nd generation antihistamines in previous 72 hours.
- The child had an episode of anaphylaxis *of any cause* in 4 weeks prior to challenge.

General exclusion criteria were:

- Acute infections or allergies.
- Uncontrolled atopic dermatitis.
- Chronic urticaria or mastocytosis (including urticaria pigmentosa).
- Underlying cardiac, hepatic, renal, or other diseases, where exposure might affect patients' safety or the correct interpretation of the challenge outcome.

Outcome measures

- The primary outcome measure was level of milk tolerance achieved by 6 months post randomisation/challenge. A responder was defined as having reached an IMAP milk ladder position of step 6 or above at 6 months.
- Secondary outcomes were changes in food allergy-related quality of life (FAQL) measures from randomisation to 1, 3, 6 and 12 months post randomisation, changes in State and Trait Anxiety(STAI) scores from randomisation to 6 and 12 months.
- Changes in milk SPT and serum levels of milk specific Ig E from 0-6 months in each group.

Statistics

Sample size and power calculation

Power analysis for a logistic regression was conducted to determine a sufficient sample size using an alpha of 0.05, a power of 0.8, a large effect size (odd ratio = 2.48) and a one-tailed test. Based on the aforementioned assumptions, the desired total sample size is 55 (27). The major study showing accelerated tolerance is Kim 2011(9). It's definition of tolerance is unrestricted consumption of all unheated forms of milk, demonstrated as 0% in all children at 6 months, and 14% in baked milk tolerant children by 12 months. Our unpublished IFAAM data showed 25% (3/12 children) fully tolerant and 58% (7/12) partially tolerant on step 6 of 12 on the iMAP ladder at 6 months post single dose exposure and 66% (3/5) fully tolerant by 12 months.

Our primary hypothesis is that use of a single dose OFC would increase tolerance (as defined by reaching at least step 6) by 25% between the treated and control groups,

a large effect size. Patients were randomly assigned to 1 of 2 groups, the control group A (P_1) receiving standard care, and an active single dose group B (P_2).We carried out a power analysis to determine how many subjects would be needed to test a difference in proportions between groups A and B of 0.25 with a power of 0.80% at p=0.05. Note that these hypotheses constitute a one-tailed test, which is justified by the preliminary observation in the IFAAM group of no adverse effect/decreased tolerance of milk after the single dose intervention. Power analysis indicated that we needed N=20 subjects in group A (P_1) and N=40 subjects in group B (P_2) to find a change in probability of 0.0001 with a power of 0.8 and an alpha < 0.05.

This project was assessed by UCC's Office of Corporate and Legal Affairs (OCLA) and was considered not to be a regulatory trial, rather it was an exploratory study into the feasibility and potential of the single dosing intervention alongside routine care with the milk ladder. We therefore planned a Per Protocol analysis rather than an Intention To Treat analysis. All analysis were agreed before study enrolment.

Summary statistics were used to compare the features of the intervention and control arm patients. Logistic regression was used to examine interaction of variables of interest including age , sex, entry and exit SPT wheal size and milk-specific IgE levels.

RESULTS

Cohort Description

109 outpatient referrals with possible milk allergy to Cork University hospital and Our Lady's Children's hospital Crumlin, Dublin were reviewed. Of these 78 were screened. 17 had negative SPT and 1 had generalised urticaria and accordingly were designated screen fails.

60 cow's milk allergic infants were recruited and randomised, 40 to the intervention arm and 20 to control.

25 were recruited from CUH and 35 from Our Lady's Children's Hospital Crumlin. 57 were followed up to 6 months and 55 to 12 months post randomisation giving a completion rate of 92%. Milk ladder position (but not SPT or questionnaires) was available for 57 at 12 months as 2 patients who had been lost to follow up were subsequently contacted by phone.

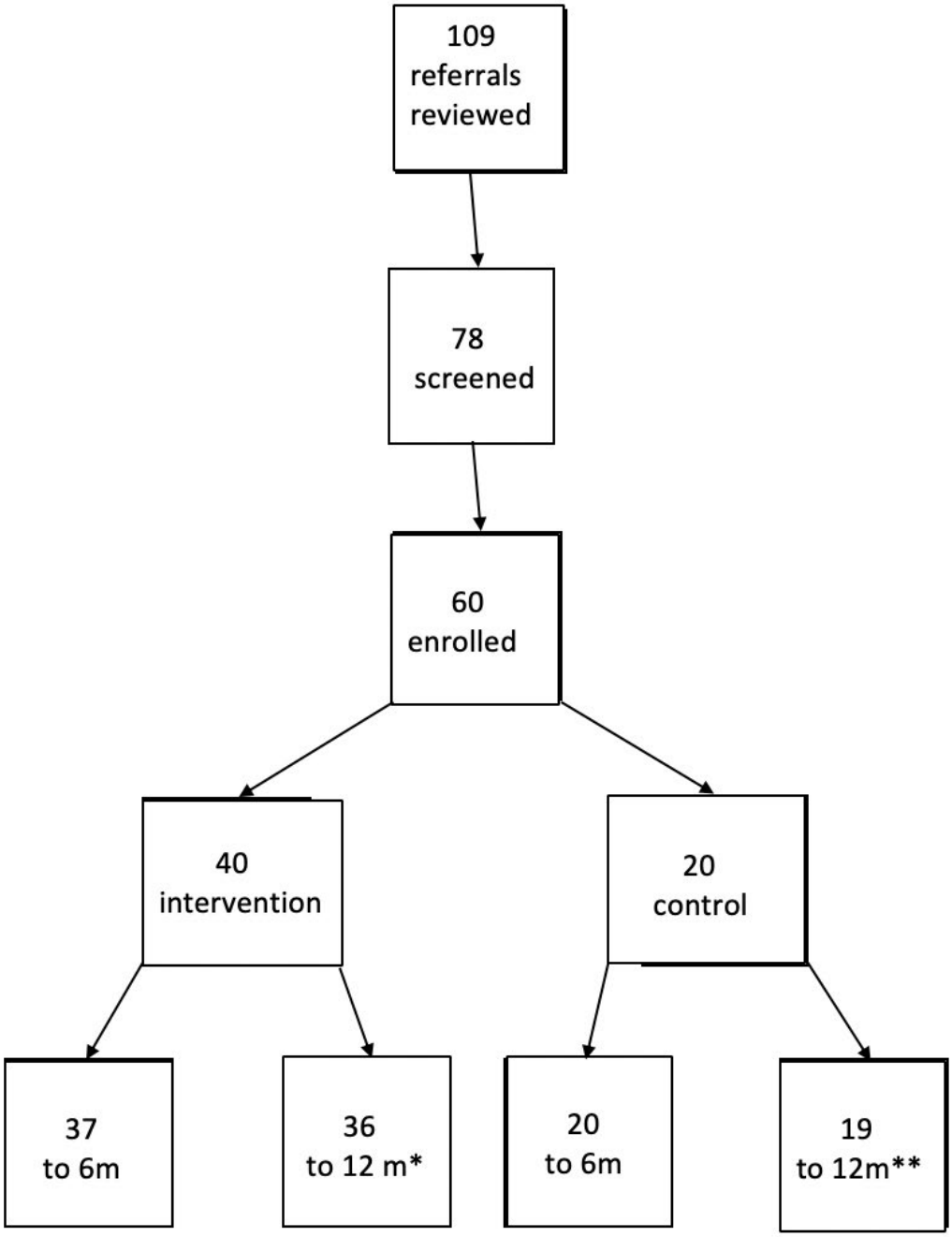

Figure 4 Study flow chart

	Intervention	Control	Total
Sex	29/40M 11/40F	11/20M 9/20F	40/60M 20/60F
Mean age (months)	6.25	8	7
Milk SPT mm (mean)	5.96	6.10	5.60
Milk spIgE (KU_A/L)	11.3	8.67	12.73
Eczema	28/40 (70%)	15/20(75%)	43/60(72%)
Egg sensitised	21/40 (53%)	19/20(95%)	40/60(67%)
Peanut sensitised	19/40(48%)	8/20(40%)	27/60(45%)
Egg and peanut sensitised	12/40(30%)	8/20(40%)	20/60(33%)
Method of feeding at appointment	39/40(98%) breastfed	20/20(100%) breastfed	59/60(98%) breastfed
Anaphylaxis at presentation	1/40(2.5%)	0/20(0%)	1/60(1.7%)
Days since last reaction (mean)	20.5	27.5	29.5

Table 1 : Patient Demographics

There were 4 reactors to the single dose challenge. All four reactions were mild. 2 babies had facial hives, one had lip swelling and one had immediate vomiting. All reactions occurred within a few minutes of administration of the 1-shot and resolved spontaneously within 30 minutes. No treatment was necessary in any of the cases.

The 4 reactors were all male, SPT 5-6mm, spIgE available for 2 were similar (1.36 and 1.48). They varied in age from 5.5 to 11.5 months, had similar initial reactions to dairy products but sensitization to other foods varied from no other sensitization (1), sensitized to egg only (1) or sensitized to egg and peanut (2). Despite having reacted to the ED05 of milk, all 4 of these children progressed rapidly up the milk ladder. All were on step 9 or above by 6 months and all had reached step 12 achieving full tolerance by 12 months post randomization.

Study ID	Age (M)	Sex	Previous reaction	SPT mm	Milk SpIgE	Eczema	FHx Atopy	Other food sens	1-shot reaction	1-shot outcome	6m MLP	12m MLP
026	6	M	Rash,lip swelling	6mm	1.36	N	Y	No	Lip Swelling	Resolved No treatment	Step 10	Step 12
029	11.5	M	Rash, Vomit	5mm	N/A	Y	N	Egg, peanut	Hives on Face	Resolved No treatment	Step 10	Step 12
036	8	M	Hives, Rash	6mm	N/A	Y	N	Egg, Peanut	Hives on face	Resolved No treatment	Step 9	Step 12
050	5.5	M	Hives, Rash, Vomit	5mm	1.48	Y	Y	Egg	Vomit	Resolved No treatment	Step 12	Step 12

Table 2 . 4 children reacted to the single dose challenge

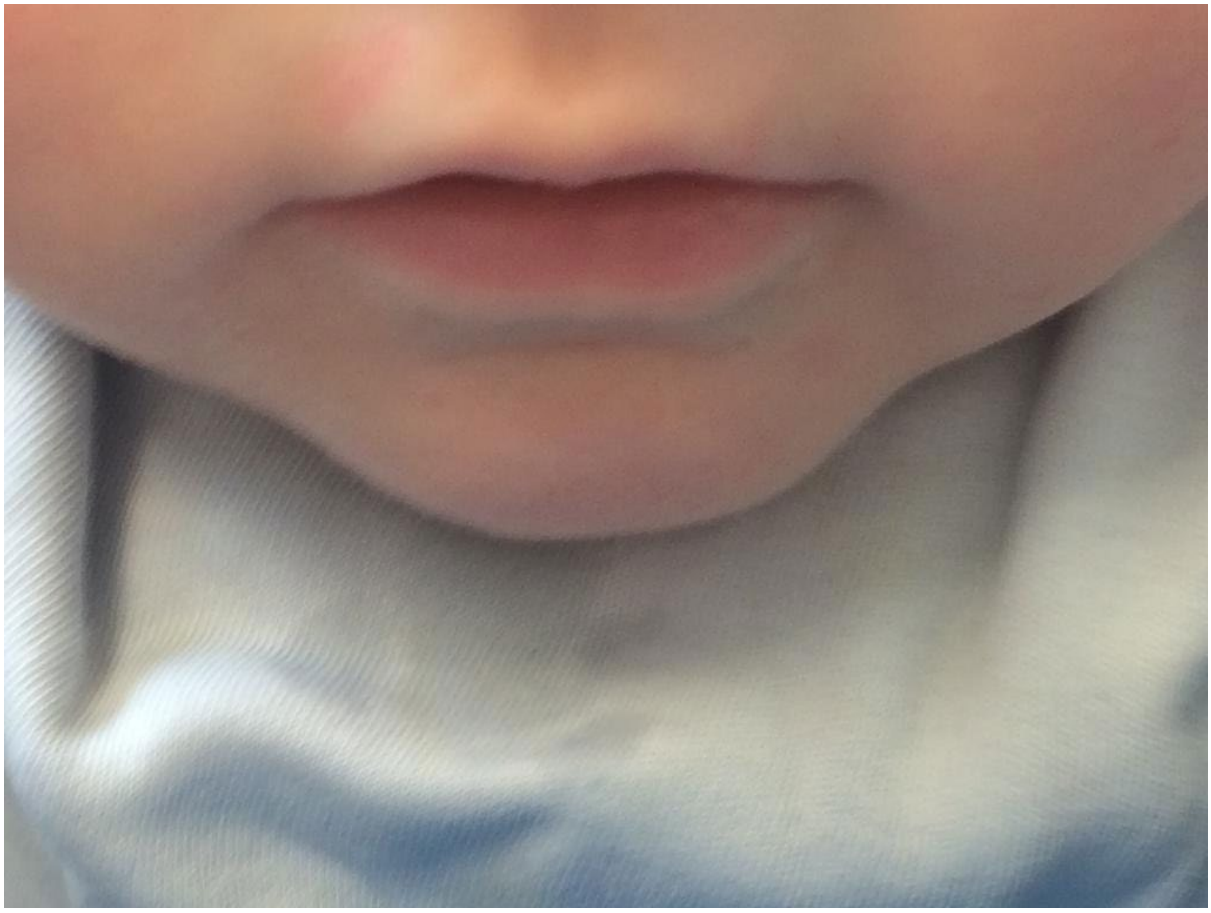

Figure 5 Mild perioral urticaria after single dose of milk ED_{05} (subject 029)

Adverse events

During the course of the study there were no SAEs. 1 child was admitted to A&E for observation for 6 hours because of croup, unrelated to the study.

2 patients had accidental exposures to milk with reactions at steps above their current level on the milk ladder. 1 of these had 2 accidental exposures at creche, one of which resulted in attendance at A&E and treatment with antihistamine. The other child had

an accidental exposure in a relative's house and was treated with antihistamine at home.

There were no unexpected or serious adverse reactions reported while progressing correctly up the milk ladder.

Appointment loss due to COVID-19 restrictions

6 follow up appointments at 6 months and 13 at 12 months were done virtually due to Covid 19 restrictions, leading to missing blood samples and SPT results for these participants.

Milk ladder position.

The primary outcome measure for this study was Milk Ladder Position (MLP) at 6 months post recruitment. Step 6 (lasagne) is halfway up the milk ladder and was selected as a step most likely to be achieved at 6 months that would be clinically meaningful. This is because of the young age of babies at recruitment, they had only started weaning. Step 12 is being able to fully tolerate unheated milk.

A child was designated a "responder" if he/she was at step 6 or above at 6m after randomisation. A child was designated a "non-responder" if he/she was at step 5 or below at 6 months.

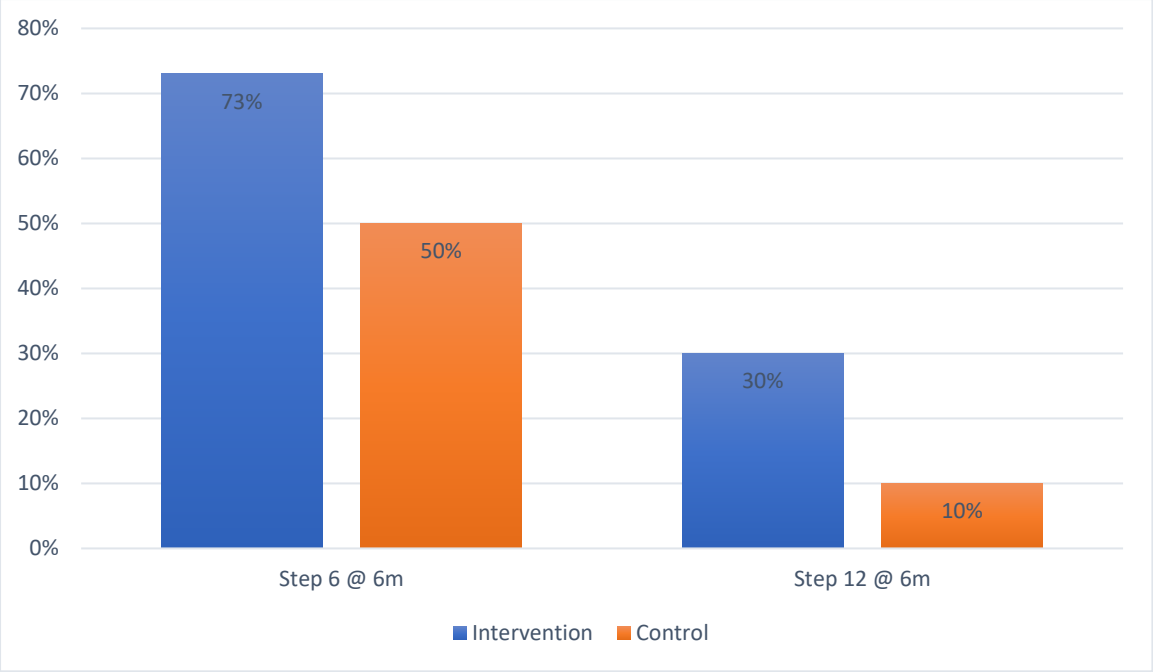

Figure 6 Milk Ladder Position at 6 months

At 6 months post randomisation 27/37 (73%) infants in the intervention group were on step 6 or above of the milk ladder compared to 10/20 (50%) in the control group. There is a 23% absolute difference between the intervention and control groups in achieving step 6 after 6 months (p= 0.048).

11/37(30%) of the intervention group hadreached step 12 compared with 2/20(10%) of the control group at 6 months.There is a 20% absolute difference between the groups in reaching step 12 after 6 months (p=0.049). The number needed to treat is 3.7.

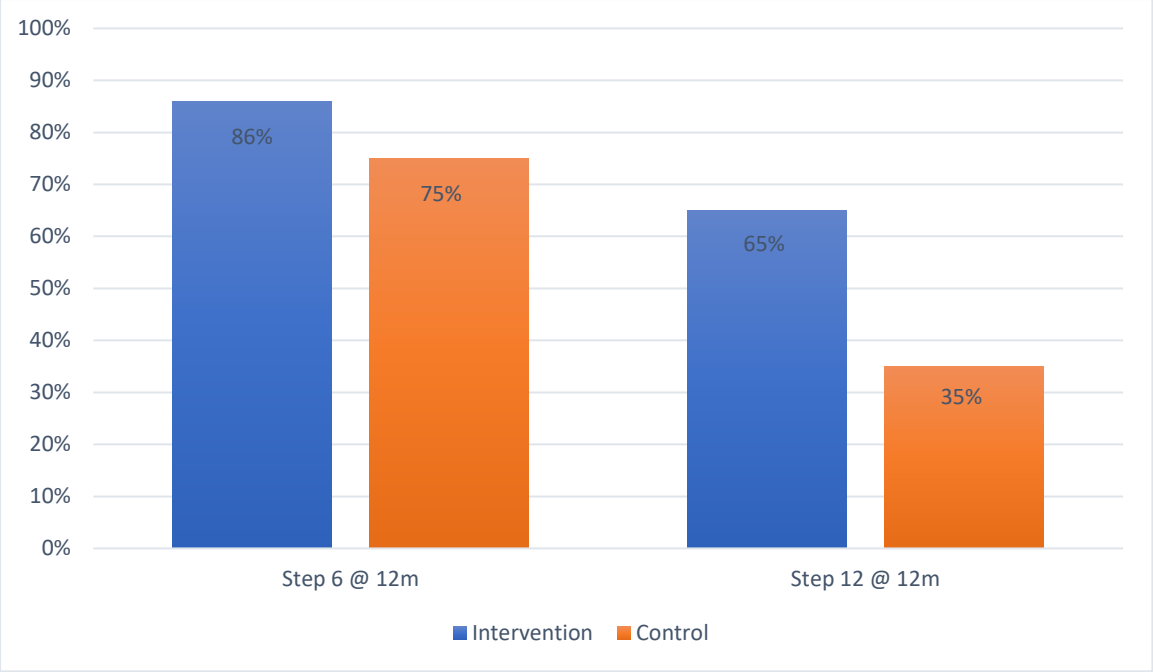

Figure 7 Milk Ladder Position at 12 months

Overall as a group, 37/57(65%) were on step 6 at 6 months, and 13/57(23%) were on step 12 at 6 months. 47/57(82%) were on step 6 at 12 months and 31/57(54%) were on step 12 of the milk ladder 12 months after randomisation.

At 12 months post randomisation 32/37(86%) of the intervention group and 15/20(75%) of the control group were on step 6 or above (chi sq =1.18, p =0.27). However, significantly more of the intervention group 24/37(65%) has completed the ladder (step 12) compared to just 7/20(35%) of the control group (chi sq 4.7, p =0.03).

Relation of baseline SPT and SpIgE with responder status at 6 months and 12 months

Baseline SPT was significantly associated with responder status at 6 months (baseline SPT for responders 5.7mm vs 6.7mm in non responders) but not 12 months. Baseline milk spIgE was also significantly associated with responder status at 6 and 12 months (baseline milk spIgE for responders 4.7KU$_A$/L vs 37.5 for non responders).

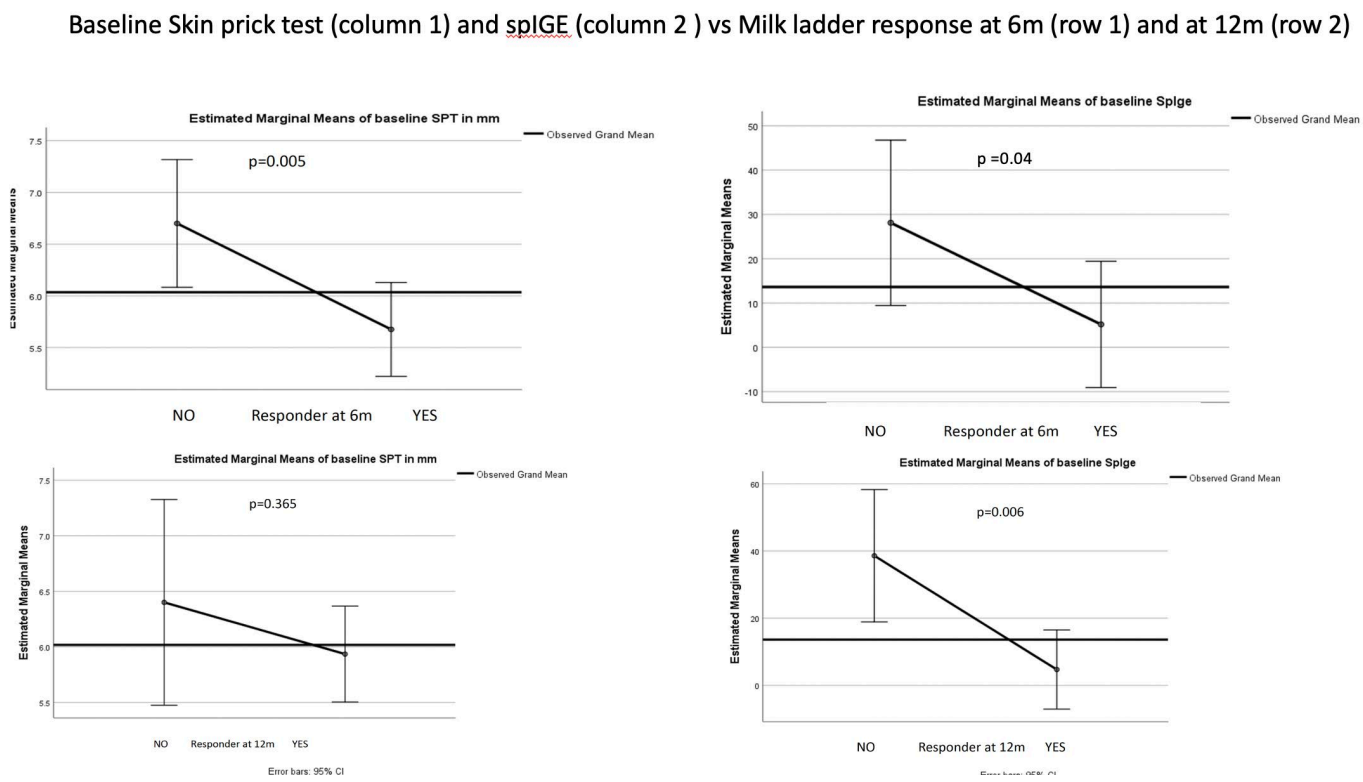

Figure 8 Baseline milk SPT and milk spIgE levels are associated with responder status at 6 and 12 months

Progress on the milk ladder has a significant impact on SPT, with a significantly larger decrease for responders (at step 6 or above) vs non-responders (below step 6), irrespective of treatment group (ED_{05} single dose given or not given). This effect was greater in the treatment group (who received milk ED_{05}) than control group (no ED_{05}

dose): responders in the treatment group, baseline SPT(M=5.6, SD=1.6) and at 6 months SPT (M=2.3, SD=2.0); [t(23)=--10.63, p = 0.0001]. For responders in the control group, (M=5.5, SD=1.4) and at 6 months (M=3.8, SD =2.2); [t(9)-3.43, p=0.008).

Maternal State and Trait Anxiety

A repeated measures Analysis of Variance was used to investigate change in scores over time for the whole group, irrespective of randomisation. There was a statistically significant effect of time on State-Anxiety (S-Anxiety), [F= 4.85, p = 0.002] Figure. In contrast, maternal Trait anxiety (T-anxiety) scores did not change significantly over time [F= 0.67, p = 0.6].

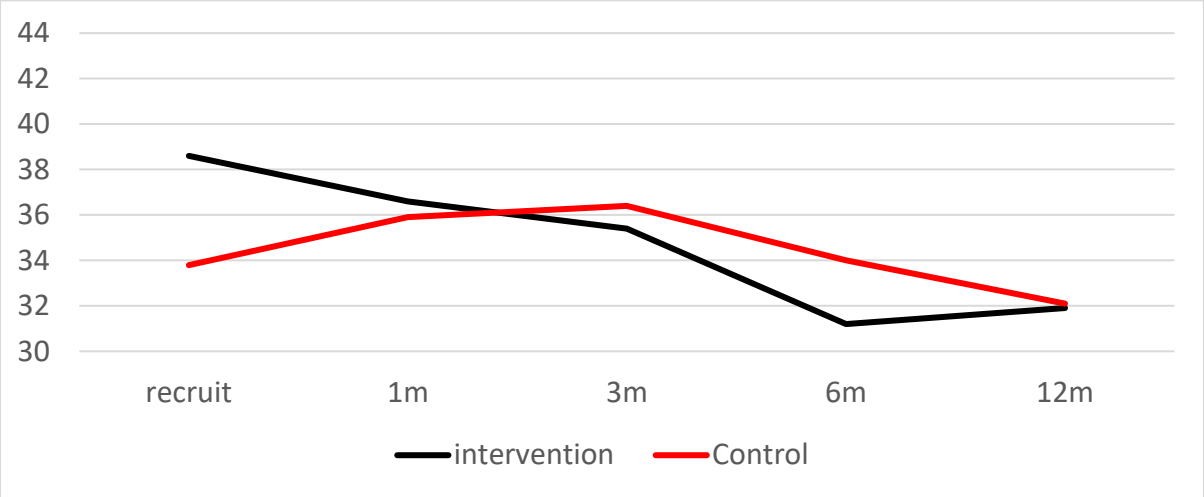

Figure 9 Changes in State Anxiety scores over time per group

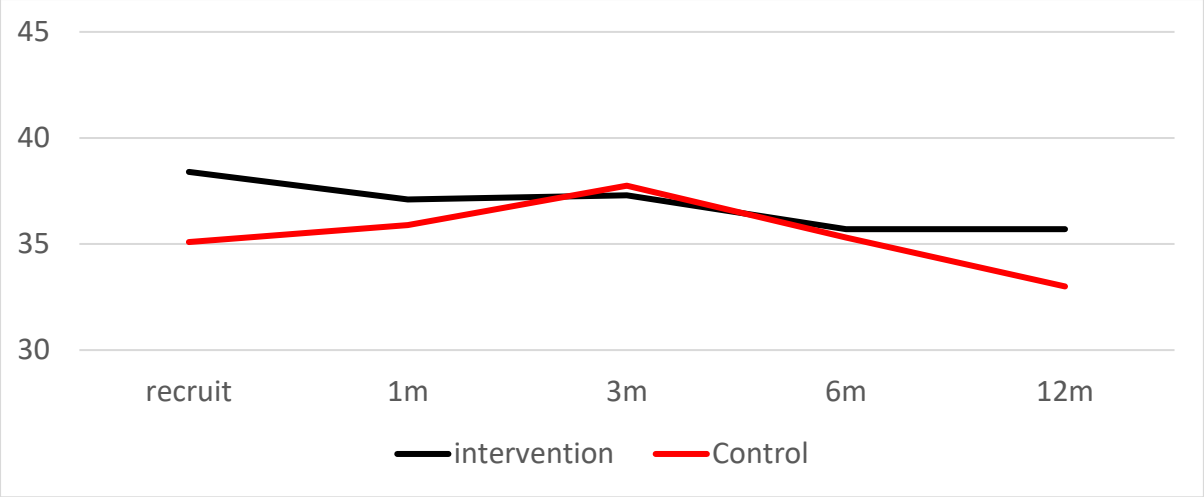

Figure 10 Changes in Trait Anxiety scores over time per group

Effect of treatment outcome on maternal State and Trait anxiety

We carried out an analysis of covariance (ANCOVA) to investigate any differences in Anxiety at 6 months, for Group (treatment vs control) and Response status (responder vs non-responder), controlling for S-Anxiety and SPT in mm, at baseline.

Main effects for Group [F(47) =0.235, p=0.63) was not significant, meaning the groups were balanced at baseline. The main effect for Response status [F(47) = 4.751, p=0.035] was significant, indicating that scores on S-Anxiety at 6 months differed significantly for responders vs non-responders, controlling for baseline S-Anxiety score. This means anxiety improved from baseline to 6m if the baby was a responder to treatment, ie. at step 6 or above.

Means and standard deviations for responders vs non-responders shows S-Anxiety decreased from a mean of 37.9 (SD=12.7) at baseline to 32.7 (SD=9.9) at 6 months for responders, and for non-responders from 33.2 (SD=8.9) to 31.1 (SD=10.1), baseline to 6 months, respectively, whether they were a responder in the control group or a responder in the treatment group. However, being in the treatment group

conferred a greater advantage in terms of improvement, i.e. those in the treatment group who were responders improved MORE than those in the control group who were responders, but both improved).

Treatment vs Control for State- and Trait-Anxiety

For the treatment group overall (who received the single dose of milk ED_{05}), there was a significant difference in S-Anxiety between scores at baseline (M=37.5, SD=12.9) and at 6 months (M=31.5, SD=8.6); [t(32)=--2.81, p = 0.008]. For the control group overall (no ED05 dose), no significant difference was found in S-Anxiety for scores at baseline (M=33.1, SD=8.5) and at 6 months (M=31.7, SD =11.6); [t(14)4.17, p=0.59).

For the treatment group, no significant difference in T-Anxiety was found between scores at baseline (M=37.5, SD=10.4) and at 6 months (M=36.2, SD=8.6); [t(31)=--1.01, p = 0.32]. For the control group, no significant difference was found in T-Anxiety for scores at baseline (M=34.7, SD=9.1) and at 6 months (M=35.1, SD =9.1); [t(14)0.21, p=0.59).

These results suggest that treatment (the single dose of milk ED_{05}) has a significant impact on level of maternal State- anxiety. State anxiety reduces from baseline to 6 months for treatment cases (whole group) but not for control cases (whole group). In contrast treatment has only a marginal but not significant impact on level of Trait-anxiety, supporting the paradigm of state anxiety being temporary/modifiable but trait anxiety being stable/not easily modifiable over time, due to stable factors/”traits”.

Responder vs Non-responder for State- and Trait-Anxiety

For the treatment group, there was a significant difference in State-Anxiety between scores at baseline (within group) for responders (at step 6 or above) vs non responders (below step 6) (M=37.9, SD=12.8) and at 6 months (M=33.2, SD=10.5); [t(30)=--2.23, p = 0.03]. For the control group (within group), no significant difference was found in State-Anxiety for scores between baseline (M=32.8, SD=89.0) and 6 months (M=28.6, SD =6.9); [t(16)-1.47, p=0.16) These results suggest that responding to treatment has a significant impact on level of state anxiety. Specifically, our results suggest that the level of state anxiety reduces from baseline to 6 months for those who respond to treatment in either the intervention or control group.

A paired-samples t-test was conducted to compare Trait-Anxiety at baseline and 6 months, split by responder vs non-responder status, showing no effect in either group. For the treatment group, baseline (M=37.8, SD=10.5) and at 6 months (M=36.9, SD=8.6); [t(29)=--0.615, p = 0.54]. For the control group, scores at baseline (M=34.5, SD=8.8) and at 6 months (M=33.8, SD =8.6); [t(16)0.48, p=0.64).

Response to treatment with ED_{05} has a marginal but not a significant impact on level of Trait anxiety but State-Anxiety decreased significantly for responders in both treatment and control groups.

Baseline Anxiety Score (tertile)	SPT (mm)	SpIgE
Low trait	6.23	11.2
Med trait	5.81	17.5
High trait	5.9	14.1
Low state	6.5	21.2
Med state	5.7	17.3
High state	5.7	2.6

Table 3 Baseline SPT vs Trait and State anxiety tertiles.

There was no correlation between baseline maternal trait and state anxiety scores and baseline SPT or spIgE. However maternal anxiety scores were significantly associated with changes in SPT from baseline to 6 months and to 12 months. This effect was more evident within a treatment group and when subjects were split according to being a responder or non-responder at 6 months. Similar effects were seen for SpIgE (data not shown)

Trait anxiety tertile	Change of SPT Baseline-6mo (mm)	Paired samples t test p
Low (<30)	2	0.011
Medium (30.1- 44.9)	2.7	<0.001
High (>45)	1.3	0.04

Table 4 Baseline trait anxiety tertiles vs change of SPT 0-6m

Trait anxiety tertile @6m	Responder Status R/NR	Change of SPT 0-6m (mm)	Paired samples t test p
Low	R	-3.2	0.003
	NR	-1.5	0.21
Medium	R	-3.6	<0.001
	NR	-1.8	0.05
High	R	-2.5	0.03
	NR	+1.0	0.5

Table 5 Trait anxiety vs change SPT 0-6m according to responder status (intervention group only) (similar pattern in control group, data not shown)

Tabkle 5 shows significant decreases of SPT in all responder groups from 0-6 months. The only group to show an increase in SPT was the high anxiety non-responder group. As trait anxiety is stable over time, this effect is probably not due to reverse causation (which would be that an increase of SPT in a non-responder increases trait anxiety).

State anxiety vs change in SPT 0-6m acc to responder status (all patients)

State anxiety tertile @6m	Responder Status R/NR	Change of SPT 0-6m (mm)	Paired samples t test p
Low	R	-3.1	<0.001
	NR	-1.3	0.07
Medium	R	-3.0	<0.001
	NR	-1.0	0.275
High	R	-2.0	0.012
	NR	-0.5	0.5

Table 6 State anxiety vs change of SPT 0-6m, responders only (both groups)

All responders showed significant decreases in SPT, non-responders showed no significant change. The “high state anxiety” non-responders showed the least change. Findings were similar for trait anxiety at 6m (data not shown).

The findings relating to anxiety tertiles, changes in SPT in responders and non-responders were also consistent out to 12m, with the only group to show an increase in SPT again being the “high state anxiety” non responders.

State anxiety tertile @6m	Responder Status R/NR	Change of SPT 0-12m (mm)	Paired samples t test p
Low	R	-3.2	<0.001
	NR	-0.5	0.83
Medium	R	-4.75	<0.001
	NR	-0.67	0.78
High	R	-3.8	0.028
	NR	+1.5	0.21

Table 7 State anxiety vs change in SPT 0-12m acc to responder status (all patients)

FOOD Allergy Quality of Life and FAIM.

FAQL and FAIM scores were similar in each group at baseline.

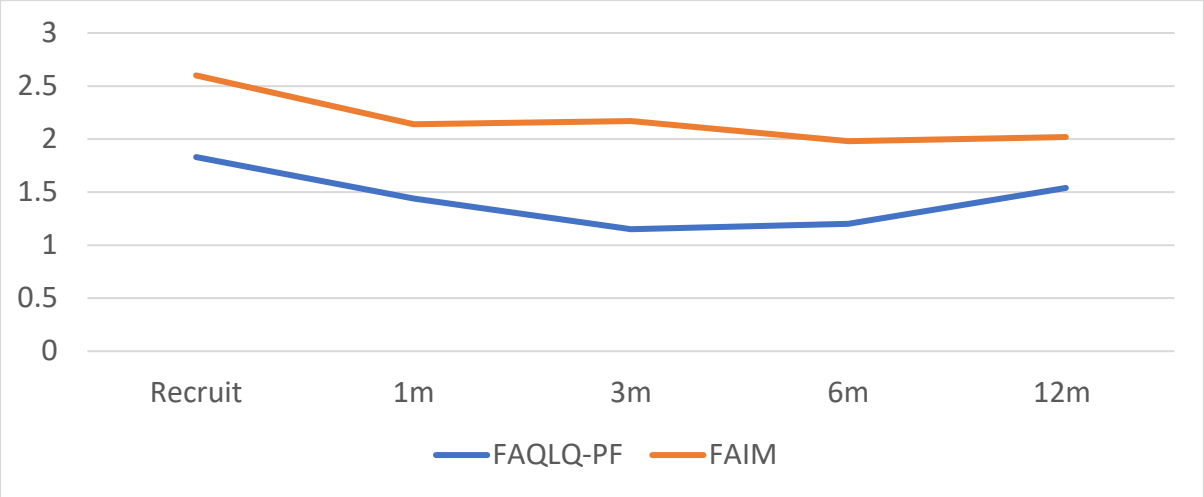

Figure 11 Changes in FAQLQ (PF) and FAIM scores over time (whole group)

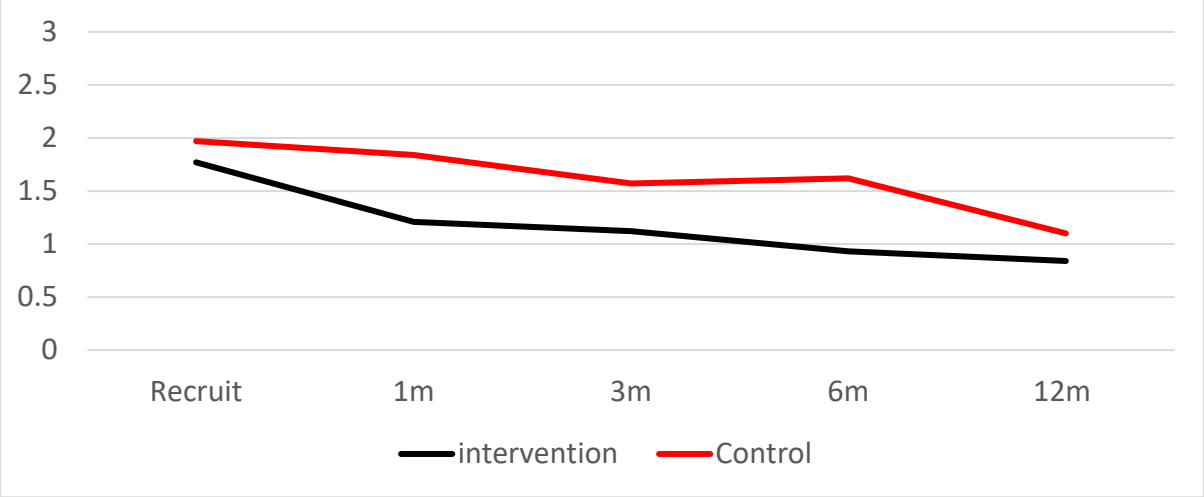

Figure 12 Changes in FAQLQ (PF) scores over time per group

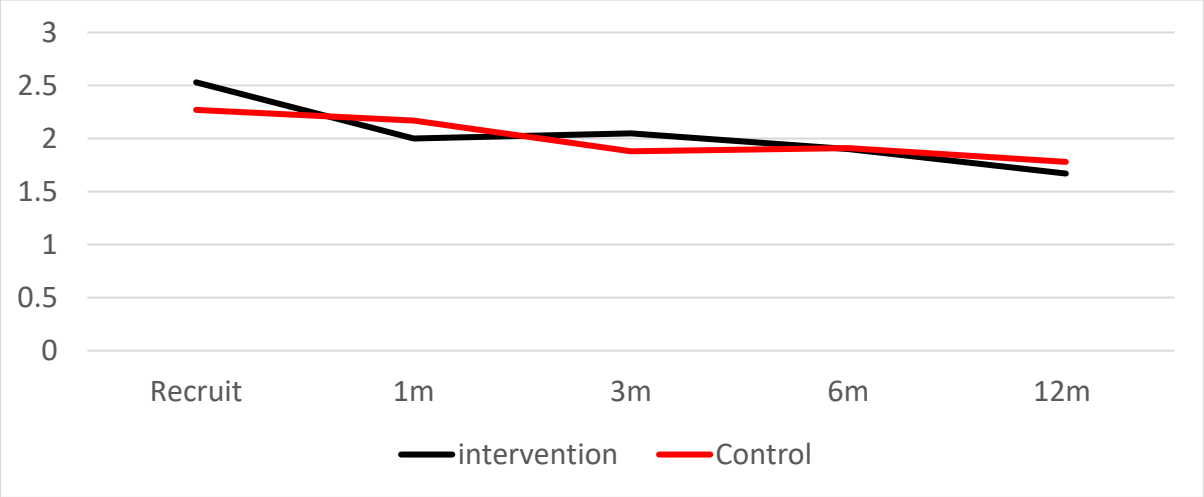

Figure 13 Changes in FAIM scores over time per group

Treatment group	Responder non responder (R/NR)	FAQLQ baseline	FAQLQ 6m	FAQLQ 12m
Intervention	R	1.85	0.94*	0.53*
	NR	1.1	1.45	0.71
Control	R	1.51	0.8	0.85
	NR	2.2	1.1	0.97

(* for change from baseline value $p < 0.05$)

Table 8 FAQL scores in 6m responders at baseline, 6m and 12m

FAQL score improved more than the minimum clinically important difference (MCID) in all but one group by 6m and in all groups by 12 months. Changes in FAQL were statistically significantly different between responders and non-responders in the intervention group only.

FAIM scores improved more than MCID in both groups over time in a similar fashion but this again only reached statistical significance in the intervention group responders at each time point, 6m and 12m (data not shown).

DISCUSSION

We undertook this study to formally evaluate a clinical impression that was formed after the conclusion of the IFAAM single dose study. This impression was that the children involved were achieving tolerance to cow's milk using the milk ladder relatively quickly. We wanted to determine if this was a real effect and to investigate potential reasons. We also wanted to determine if starting the milk ladder in very young infants, immediately after diagnosis is safe and effective.

Primary outcome

Our primary outcome for this randomized study of 60 cow's milk allergic infants was to compare the acquisition of tolerance to milk between the intervention and control groups at 6 months after randomisation. We showed a statistically significant difference between the 2 groups for both endpoints with a 23% absolute difference between the groups in getting halfway up the ladder (step 6) and a 20% absolute difference in achieving full tolerance (step 12).

After 12 months there is also a statistically significant difference between the 2 groups in reaching step 12 on the ladder. Most infants in both groups are at least half way up the ladder at this point but in the control group there is a lack of progress to the final step which is feeding unheated milk to the infant. This may be due to parental anxiety as giving the child 'real milk' can be interpreted as more dangerous than the previous steps. It may be that the parent who has already experienced their child receiving milk in the form of the single dose challenge has more confidence to progress to this last stage.

As was expected, the maternal trait anxiety remained constant throughout and the state anxiety changed over time. This could have implications for the future management of cow's milk allergic infants and possibly children with other food allergies, especially egg allergy as this is one of the other common childhood food allergies and is treated in Ireland in a similar way by using the egg ladder. It may be possible to identify the most anxious parents (ie. those most at risk of not progressing) and to either use the single dose challenge as a simple way to provide reassurance or to put in place extra support to help these particular patients progress up the ladders. This could be a simple intervention such as regular telephone support calls from the allergy team.

We had a balanced randomization sample with no significant difference in age, incidence of eczema, feeding method, time since last reaction between the intervention and control groups. We did note that there was an incidental finding of a higher rate of egg sensitization in the control group (95% compared to 53% in the intervention group) , however this may be balanced somewhat by the higher rate of peanut sensitization in the intervention group (48% compared to 40% in the control group).Sensitisation to one food is not known to inhibit natural or induced tolerance to another allergen.Both groups were treated with the same care plan (the milk ladder) and thereforenthere is unlikely to have been any effect of egg sensitization on milk tolerance acquisition.

Safety data

Safety of single dose challenge

This study has reinforced data from previous studies (18,19) which have shown that the single dose challenge is a safe procedure. There were 4 reactors from the 40

single dose challenges performed in my MSc study. The reactions were classed as positive as all 4 met the objective criteria for a positive challenge as set out in the previous IFAAM study. All reactions occurred within a few minutes of administering the single dose and all had resolved spontaneously without any treatment within 30 minutes (Table 2). There were no late phase or secondary reactions. There was no single factor identified which could predict reaction to the single dose when compared to the group as a whole. It is interesting to note that even though these infants reacted to the single dose challenge all of them progressed rapidly up the milk ladder achieving full tolerance by 12 months post randomization.

Safety of milk ladder protocol

All 60 infants in this study were started on the milk ladder at diagnosis regardless of age or severity of initial reaction, including the infant with a history of anaphylactic reaction to milk.It is of interest to note that this child progressed on the ladder without incident to achieving full tolerance. All were at the age of weaning onto solid food so it was possible to start immediately. There were no serious or unexpected reactions progressing up the ladder. It is to be expected while using the ladder that some children will have mild reactions when transitioning to a higher step on the ladder. In routine clinical practice, if this happens the parent is advised either to keep their child on the current step and try to transition again after 7-10 days or if the reaction is very mild (for example 1 or 2 hives or some redness around the mouth) to continue at the higher step unless symptoms become more severe. There were no serious reactions which would have led to stopping the milk ladder reported by the parents of any of the 60 infants in this study. There was no requirement for use of adrenaline autoinjectors

throughout the study. These are very important safety data with regard to use of the milk ladder in young infants. I have shown that it is safe to start baked milk from a young age (ie. immediately at diagnosis), regardless of the severity of the initial reaction. This data correlates with data on home introduction of baked egg from a recent study in CHI-Crumlin (28). In this report, 23 referrals for egg allergy were reviewed and 22 contacted by telephone. 21 were advised to introduce baked egg at home, 1 was excluded due to anaphylaxis to egg. 4 weeks later 76% had successfully introduced baked egg at home. 18% felt confident to introduce egg but hadn't due to perceived contraindications (eczema,illness). This reinforces the safety data for home introduction of both milk and egg at diagnosis in the form of baked milk and egg.

3 accidental exposures were reported during the course of the study. These incidents remind us of how vulnerable these infants are when in the care of others and the worry this can cause their parents.

Outcome and efficacy of procedure

We have shown that there is a statistically significant difference in outcome between the intervention and control groups in this study. At 6 months 73% of the intervention group were at step 6 compared to 50% of the control group and 30% were at step 12 compared to 10% of the controls.12 months post randomisation 65% of the intervention group were tolerating unheated milk compared to just 35% of the control group. This shows us that the intervention of the single dose does affect the outcome in these infants helping them to achieve tolerance to milk at a faster rate.

The results for the intervention group are very similar to, if not better than, our observations in the original IFAAM study where 14% were tolerant of unheated milk at 6 months and 58% at 12 months post single dose.

However, in this study even the control group have outcomes better than expected. In the study by Kim et al (9) no child was tolerant of unheated milk after 6 months and only 14% were tolerant of unheated milk after 12 months. This is a substantial difference when compared even to to our control group who were using the milk ladder, where 10% were consuming unheated milk after 6 months and 30% after 12 months.

Even without the single dose, use of the milk ladder accelerates tolerance. Administration of a single dose (at the ED_{05} in this study) significantly accelerates this even further. This is unlikely to be an immunological effect as these children are by definition already allergic and have already been exposed to cow's milk by nature of their inclusion in this study. Further exposure to the ED05 of milk is unlikely to have further adverse immunological sequelae. These are very young infants and so it is more likely to relate to the confidence of the parent who is following the milk ladder and perhaps their levels of anxiety which can influence how these children progress on the ladder.

Affects of anxiety- State and Trait and FAQL

I have shown for the first time that maternal anxiety is associated with progress or lack of progress on the advised tolerance induction programme for cows milk allergy, which, in my study, was based on home use of the 12 step IMAP milk ladder. Trait anxiety levels, which are stable over time, were associated with responder status in both groups, with poor outcomes in children whose mother had higher trait anxiety

levels. State anxiety was also linked to responder status. Anxiety scores were also linked to the degree of changes in SPT and SpIgE levels, which are directly associated with degree of resolution of milk allergy - SPT and spIgE levels usually decrease as milk allergy resolves. This is an important and novel finding. We can discount reverse causation that knowledge of their child's SPT at 6m and 12m or spIgE at 6m affected the mothers' anxiety scores as the questionnaires were completed before the results of SPT were known and spIgE results were not available immediately. Admittedly mothers would have been aware of their child's position on the milk ladder before each appointment, but not of the SPT value.

FAQL and FAIM scores also tracked strongly with responder status. This is an expected outcome as, for infants of the age we studied, the FAQLQ-PF and FAIM measures a parental perception of a child's quality of life and of the parent's expectation of outcome of future allergic events. So the mother of a child who has demonstrably made significant progress with or even finished the milk ladder is likely to perceive her child as safer and more "normal".

Implications for clinical management of cow's milk allergy

I have shown in this study that the milk ladder is a safe and effective way of treating IgE mediated cow's milk allergy in infants. It is a safe way to approach home introduction of milk and can be started at diagnosis regardless of age. Currently Ireland is the only country where the milk ladder is started in young infants at diagnosis and this is mostly done only after being seen in a paediatric allergy clinic. As referenced earlier, it is also used in the UK and Australia but only after consultation in a specialist allergy clinic and at a much older age. As we have shown safety for these 60 infants I

would advocate for wider, routine use of the milk ladder at diagnosis. If patients were screened by phone it could be started at home before attendance at clinic. As a GP I would also encourage the use of the milk ladder in the community, before hospital appointment, in suspected cases of milk allergy apart from where anaphylaxis to milk is suspected. GPs with an interest in allergy or community dieticians could advise on use of the milk ladder. Further training in allergy diagnosis and use of the milk and egg ladders for community professionals who have contact with young children and their families such as practice nurses and public health nurses could be provided easily through online or face to face meetings. This would avoid delays in starting the milk ladder and so accelerate further the road to tolerance for these infants.

I have shown in this study that the single dose of the ED_{05} for milk is both safe and effective in the management of cow's milk allergy (as it augments progress on the milk ladder. It may not be not necessary to use it in every case of CMPA in infants, however it could be used as a tool in the management of cases where progression on the ladders is proving difficult. It could be used as a simple and safe intervention to provide reassurance to parents and help them overcome some of their anxieties about their child's diagnosis and treatment. As I have shown that this is a very safe procedure, this approach could also be exported from the involved academic centres to other centres.

Following on from this study it would be interesting to further look at the role of parental anxiety in childhood allergy and methods of alleviating this which could help in the management of their child's allergic disease. This could take the form of a similar single dose study where the participants are randomised on the basis of parental anxiety scores at baseline and record if use of the single dose accelerates tolerance in the children of the most anxious parents to a similar level as that in the less anxious

parents without the single dose. It would also be interesting to have another group where the parents have a simple psychological intervention such as CBT (28) to determine whether this too would promote acquisition of tolerance

Limitations and strengths of this study

There are 2 main limitations of this study. Firstly there was no placebo group. This was primarily because we wanted to compare the intervention of the single dose with our current normal standard of care which is introduction of the milk ladder after diagnosis without an OFC. Addition of a placebo group would have necessitated recruitment of at least a further 20 infants to the study. In the IFAAM study in Cork it took 16 months to recruit 25 suitable milk allergic children of all ages and it was obvious to the research group as we planned this study that due to the time frame and funding we had for recruitment in this study, recruitment of 80 or more infants would not have been achievable.

Secondly I was not blinded as to whether the patients were in the intervention or control arm. This was due to a limitation of resources. There was only a single research fellow involved in this study and the resources and expertise were not available to have another researcher randomise and administer the single dose. This meant that I singlehandedly screened, recruited, administered the single dose challenge and did all the follow up visits.

However having a single researcher was also a strength. All appointments were completed by the same researcher leading to a uniformity in all study based

procedures such as SPT, administration of questionnaires and advice given on use of the milk ladder during the course of the study.

There was unselected, rigorous screening of participants. Recruitment was based on standard clinical criteria already shown to be effective in a previous study (18).

Another advantage to this study was that participants had their care in a local facility as much as was possible. I travelled regularly between the 2 study sites for recruitment and follow up appointments. Participants who came from areas outside of Dublin or Cork could choose which site was easier for them to access.

The participants also had other allergy care provided during the course of the study as needed, as this researcher is fully trained in allergy provision. This meant that other food allergies such as egg and peanut allergy and also eczema care could be managed within the initial and follow up appointments resulting in fewer appointments for the families and therefore less time off for work, travel, provision of childcare for other children etc as is normally needed for hospital appointments.

Conclusions

At the end of this study I can conclude that use of the milk ladder is safe even in very young children and even in infants who have reacted to the milk ED_{05}. Using the milk ladder immediately after diagnosis accelerates natural tolerance induction and a supervised single dose accelerates this further. This is most likely due to giving the parents the confidence to proceed. Some mothers of cows milk allergic children have anxious personalities (long term and short term) and this anxiety is associated with poorer progression up the milk ladder and smaller changes in skin prick test values

over time. Maternal state anxiety did predictably improve if a child was a responder but trait anxiety, which is stable and does not change greatly over time, was also associated with poorer response to milk ladder implementation and IgE related measures of reactivity (SPTand spIgE levels).

Wider adoption of early use of the milk ladder, supported by the use of the validated single dose challenge could lead to earlier resolution of CMPA in a large cohort of milk allergic infants with all of the advantages associated with early resolution. Maternal anxiety must be taken into consideration when assessing treatment plans for food allergic children. With these simple safe interventions (which may also be possible with egg allergy) it may be possible to achieve resolution of 2 of the most common food allergies in childhood by the middle of the child's second year of life.

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APPENDIX 1 Preparation of Milk ED_{05}

1. Milk ED_{05} is 0.5mg milk protein
2. Full fat milk is about 3.2% protein
3. 1 litre contains 32g milk protein
   100ml contains 3.2g milk protein.

   1ml contains 0.032g milk protein = 32mg

   So 0.015ml undiluted milk will contain 0.5mg milk protein.

Aim

To deliver 0.5mg milk protein in a more manageable volume

Calculation

0.8ml milk contains 25.6mg milk protein

Add 0.8ml milk to 4.2 mls sterile water = 5mls **Diluent 1**

5mls Diluent 1 contains 25.6mg milk protein

1ml diluent 1 contains 5.1 mg milk protein

Final volume

0.1mls Diluent 1 contains 0.5mg milk protein

APPENDIX 2 Patient narratives

Below are some comments made by the mothers of infants during the course of the study which reinforce our opinion that maternal anxiety is a huge issue to be considered in the management of cow's milk allergy in infants.

"If you read this (the STAI questionnaire)you will want to give me Xanax."

(negative 1-shot, step 0 at 6 months, step 0 at 12 months)

"I wish she was in the group that got the milk. I would have felt able to start the ladder then."

(control, step 0 at 6 months,step 4 at 12 months)

"I know it's my own anxiety keeping her back."

(control,step 0 at 6 months,step 0 at 12 months)

"Powerful impacts in a whole host of areas, not just her physical outcome. Thank you!"

(control,step 4 at 6 months, step 10 at 12 months)

"Thank you so much for helping us with his allergy. It's such a relief not to worry about this."

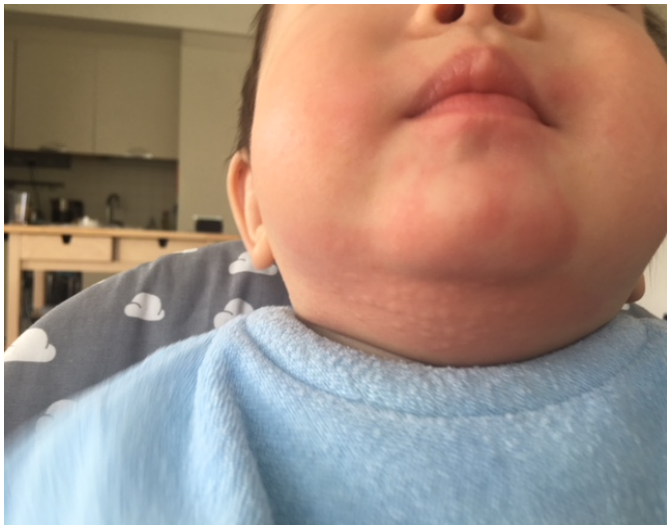
(negative 1-shot,step 12 at 6 months ,step 12 at 12 months)

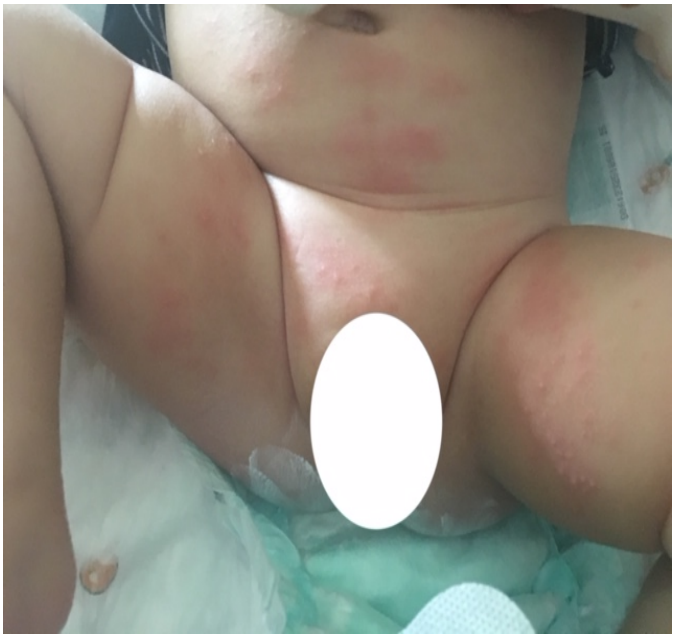
APPENDIX 3 A vignette - K's story.

K was the first infant enrolled in our study in our Crumlin site. He was 7 months old.He is a first child and was fully breast fed from birth, apart from a formula 'top up' within the first 24 hours of life while in hospital. He developed eczema at around 3 months. At 5 months he was offered some formula but developed a rash. At 7 months, on 1/4/18, he was given formula again and developed a widespread rash and urticaria. He also had a similar reaction to scrambled egg a couple of weeks after this.

I saw him and enrolled him in our study on 26/4/2018. His skin prick test to milk was 6mm. His also had a positive SPT to egg but was negative to peanut. Bloods were not obtained for spIgE. He had a negative single dose challenge and was discharged home to start the milk and egg ladders and introduce peanut into his diet.

The first steps of the milk ladder went well without reactions.However he developed a widespread urticarial rash after consuming 3/4 of a malted milk biscuit.





I advised mum to go back to giving him 1/2 of a biscuit regularly and try to increase again after a week which she did successfully. She continued with the milk and egg ladder on alternate days as she was anxious to have his allergies under control by the time she had to return to work after her maternity leave. He had no other significant reactions while progressing on the ladders.

By 1/9/18 (only 4 months after his 1-shot and 10 days before his first birthday) K was drinking formula milk and eating scrambled egg and peanut regularly with no reactions, leading to very happy parents (and baby!).