Received: 27 January 2022 | Accepted: 16 February 2022

DOI: 10.1111/all.15312

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

ORIGINAL ARTICLE

Food Allergy and Gastrointestinal Disease

Single low-dose exposure to cow's milk at diagnosis accelerates cow's milk allergic infants' progress on a milk ladder programme

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Funding information
YD was in receipt of a Clinical Research Fellowship from the National Children's Research Centre Ireland.

Abstract

Background: Cow's milk protein allergy (CMPA) is one of the most common food allergies in infancy. Most infants with CMPA tolerate baked milk from diagnosis and gradually acquire increased tolerance. Nevertheless, parents often display significant anxiety about this condition and a corresponding reluctance to progress with home introduction of dairy due to concerns about possible allergic reactions.

Objective: To evaluate the impact on gradual home introduction of foods containing cows' milk after a supervised, single low-dose exposure to whole milk at time of diagnosis.

Methods: Infants less than 12 months old referred with suspected IgE-mediated cow's milk allergy were recruited to an open-label randomized, controlled trial of intervention—a single dose of fresh cow's milk, using the validated dose of milk that would elicit reactions in 5% of CMPA subjects—the ED_{05} – vs routine care. Both groups implemented graded exposure to CM (using the 12 step MAP Milk Tolerance Induction Ladder), at home. Parents completed food allergy quality of life questionnaires and State and Trait Anxiety Inventories (STAI). Main outcome measures were milk ladder position at 6 months and 12 months post-randomization.

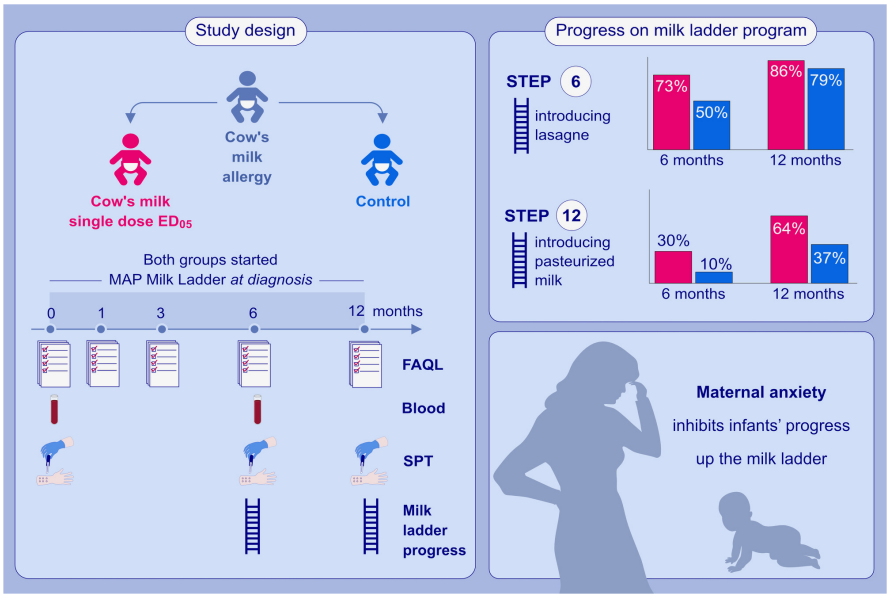
Results: Sixty patients were recruited, 57 (95%) were followed to 6 months. By 6 months, 27/37 (73%) intervention subjects had reached step 6 or above on the milk ladder compared to 10/20 (50%) control subjects ($p = .048$). By 6 months, 11/37 (30%) intervention subjects had reached step 12 (i.e. drinking unheated cow's milk) compared to 2/20 (10%) of the controls ($p = .049$). Twelve months post-randomization, 31/36(86%) of the intervention group and 15/19(79%) of the control group were on step 6 or above. However, 24/37 (65%) of the intervention group were at step 12 compared to 7/20 (35%) of the control group ($p = .03$). Maternal STAIs were significantly

Abbreviations: CHI-Crumlin, Children's Health Ireland at Crumlin; CMPA, Cow's milk protein allergy; CUH, Cork University Hospital, Cork, Ireland; ED_{05}, Eliciting Dose for 5% of subjects tested; FAQL, food allergy-related quality of life; MAP, International Milk Allergy in Primary Care Guideline; MCID, Minimum Clinically Important Difference; MLP, Milk ladder position; OIT, Oral immunotherapy; OLOLD, Our Lady of Lourdes Hospital, Drogheda, Ireland; P/NP, Progressor/non-progressor on MAP milk ladder; SAE, Serious adverse event; SOP, Standard operating procedure; SpIgE, Specific IgE; SPT, Skin prick test; STAI, State and Trait Anxiety Inventory.

associated with their infants' progress on the milk ladder and with changes in skin prick test and spIgE levels at 6 and 12 months.

Conclusion: This study demonstrates the safety and effectiveness of introduction of baked milk implemented immediately after diagnosis of cows' milk allergy in a very young cohort. A supervised single dose of milk at the ED_{05} significantly accelerates this further, probably by giving parents the confidence to proceed. Maternal anxiety generally reflects infants' progress towards completion of the milk ladder, but pre-existing high levels of maternal anxiety are associated with poorer progress.

KEYWORDS
Cow's milk allergy, immune tolerance, oral immunotherapy, quality of life, treatment



GRAPHICAL ABSTRACT

This study evaluates the impact on gradual home introduction of foods containing cow's milk after a supervised, single low-dose exposure to whole milk at time of diagnosis. Step 6 or above on the milk ladder was reached by 73% intervention subjects and 50% control subjects. Step 12 was reached by 6 month by 30% intervention subjects and 10% of the controls. Babies of mothers with higher levels of maternal anxiety at baseline made poorer progress on the milk ladder during the 12-month study period. ED_{05}, eliciting dose for 5% of subjects tested; FAQL, food allergy related quality of life; MAP, milk allergy in primary care guideline; SPT, skin prick test.

Abbreviations: ED_{05}, eliciting dose for 5% of subjects tested; FAQL, food allergy-related quality of life; MAP, milk allergy in primary care guideline; SPT, skin prick test

1 | INTRODUCTION

Cow's milk protein allergy (CMPA) is one of the most common food allergies in infancy and childhood, affecting approximately 1% of European infants[1] and can have major and lasting impacts on the affected child's physical and mental health and also on family life.[2,3] Traditionally thought to be a transient allergy, with a high rate of resolution by the age of school entry,[1] the natural history of cow's milk allergy (CMA) in populations referred to specialist centres appears to be much worse, only 6%–12% tolerant of unheated milk by around 2 years of age and 19%–75% by 4–5 years.[4,5] Oral immunotherapy for milk allergy is widely used in Southern Europe[6,7] but is onerous, usually requiring hospital supervision of dose escalation and sometimes extended inpatient stays.

There is evidence that consumption of baked milk can accelerate the acquisition of tolerance to whole cow's milk in young children.[5,8] The Milk Allergy in Primary Care (MAP) milk ladder[9] was devised to assist with tolerance acquisition in non-IgE-mediated CMPA, but some allergy centres use it in IgE-mediated allergy to facilitate the introduction of baked milk.[9,10] In the UK, for example, it is used from 12 m of age with some restrictions on home use.[11] Australia's 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, an unheated milk challenge is also performed before home introduction (personal communication Vicki McWilliam, Melbourne, Australia). As most CMPA presents around the time of weaning from breast milk, even in these 2 developed countries with well-resourced allergy services, there is clearly a large time interval

between diagnosis and initiation of baked milk introduction, which entails breast milk substitution with specialized formulas and avoidance of all milk products. In comparison in Ireland, where access to paediatric allergists is much more restricted than in UK or Australia, once the child is weaned onto solid foods and therefore able to consume the relevant foods on the ladder, the MAP milk ladder is initiated at home for both IgE and non-IgE-mediated CMPA and patients can progress without the need for multiple hospital visits. This is done irrespective of age, SPT, spIgE levels or severity of initial reaction, because it been shown to be safe[12] and is highly acceptable to families.

The single-dose oral food challenge is an efficient approach to identify the most highly dose-sensitive patients within any given food-allergic population. The eliciting dose for peanut (1.5 mg peanut protein) and milk (0.5 mg milk protein, 0.015 ml of fresh cow's milk) has been validated.[12,13] During these previous single-dose studies of ED_{05} of peanut and milk, it was evident that recruited families gained significant support and increased confidence from their participation.[13] It was also noticed that, when reviewed in routine outpatient allergy clinic after the study, the children with CMPA who had received the single-dose challenge with milk[12] were progressing to drinking whole milk relatively quickly compared to the normal, expected rate of ladder completion.

Any new diagnosis in a child can cause parents' great anxiety, and a link has also been proposed between adverse antenatal events or stressors and atopic conditions, including food allergy.[14-16] Allergists often meet parents whose anxiety appears disproportionate to their child's allergic condition[17] and many parents report living in fear of their child's death due to food allergy, when that is an extremely rare outcome.[18]

We undertook this study to formally evaluate the clinical impression that infants who received a supervised single dose of the ED_{05} of milk, had achieved both partial and full resolution of CMPA and were drinking whole cow's milk sooner than expected, irrespective of the outcome of the supervised feed. We also sought to investigate the association of maternal anxiety with their CMPA child's rate of acquisition of tolerance to cow's milk. We report here the results of a randomized trial of supervised consumption of a single low dose (the ED_{05}) of cow's milk, followed by graduated introduction of baked milk and milk products using the MAP milk ladder.

2 | METHODS

2.1 | Ethics approval

Ethical approval was obtained from the Clinical Research Ethics Committees of the Cork Teaching Hospitals and Children's Health Ireland (CHI) at Crumlin. Approval was granted on 26 September 2017. The first subject was randomized in December 2017, and the last subject's last visit was in October 2020.

2.2 | Study settings and management

Recruitment was from referrals received from primary care to 1 of the 3 centres: Cork University Hospital, Cork (CUH), CHI at Crumlin and Our Lady of Lourdes Hospital, Drogheda (OLOLD). The culture and referral practice of Irish healthcare provision means these centres already see most children with suspected IgE-mediated cow's milk allergy. The study was managed under the governance structures of the INFANT Centre (www.infantcentre.ie) UCC. Blood immunological analysis was done in Amsterdam University Medical Centers, The Netherlands.

2.3 | Study design

Infants were diagnosed with IgE-mediated CMPA by history of a recent (within the previous 2 months) typical reaction to milk or milk products and a positive skin prick test (SPT) to milk usually on same day as consent and enrolment.[12,13] SPT was performed on the volar aspect of the forearm with single tine lancets, using commercial fresh milk, cow's milk extract, and negative (saline) and positive (histamine) controls (ALK Abéllo, Reading, UK). SPT was positive if a wheal mean diameter of 3 mm to milk extract or fresh milk was seen at 15 min, in the presence of a 3 mm wheal to histamine. The same body site and equipment were used for SPT at 6 m and 12 m. A formal, double-blind placebo-controlled food challenge was not performed. Study-specific exclusion criteria were as follows: the child was already tolerating baked milk products; parental inability to give written informed consent in English; being medically unfit according to local unit guidelines/ protocol (e.g. high fever, wheeze, unwell with intercurrent illness, antibiotics in previous 14 days) and not having any intervention or event which could mask signs of an IgE-mediated allergic reaction during the supervised single dose of cows' milk: having systemically received corticosteroids within 14 days prior to randomization; used 1st generation antihistamines in previous 7 days or 2nd generation antihistamines in previous 72 hours and an episode of anaphylaxis *of any cause* in 4 weeks prior to challenge.

After written parental informed consent was obtained, children were randomized (by random number generation, in a ratio of 2:1) to intervention (single dose of 0.5 mg milk protein, followed by MAP milk ladder implementation at home) or routine care, before using MAP milk ladder implementation at home (Figure 1). Adrenaline autoinjectors were prescribed as considered necessary by the supervising clinician (JOBH, AB) and were not routinely prescribed as part of study design.

2.4 | MAP milk ladder

At the time of the study, a 12 step MAP ladder was available, with extensively baked goods such as cakes and cookies at Step 1 (see

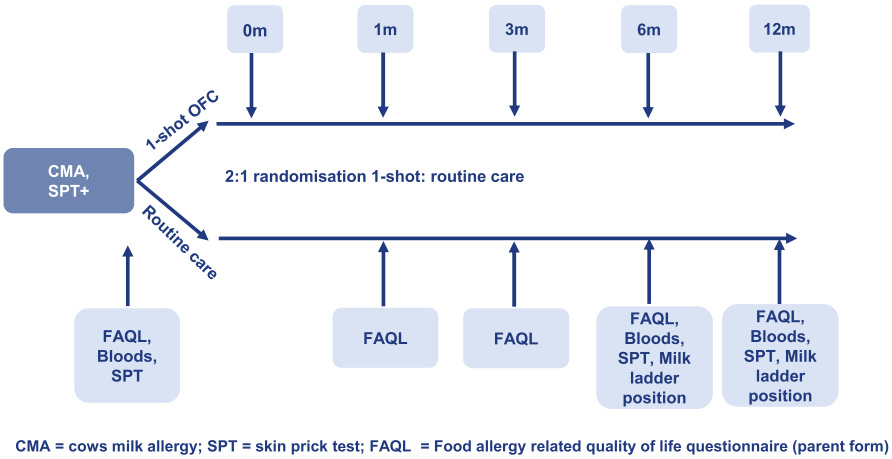


FIGURE 1 Study outline 40 children randomized to intervention arm, 20 to control arm

Appendix 1). As foods were tolerated, gradual escalation was advised, at home, to other intermediate steps involving less extensively cooked milk in foods, then yogurts, cheese and finally whole milk at step 12. Infants needed to be tolerant of a normal age-related portion of each food on at least 3 occasions in 1 week before moving up to the next step of the ladder, where a small portion size had to be tolerated before increasing portion size. Baked goods could be store-bought or home-made. Home-made products could be made with whole cows' milk, in line with Irish national policy and advice to use whole cows' milk as a cooked ingredient or as a complimentary drink before 12 months but not as a primary milk source. If infants passed 12 months of age during the study, they could drink whole cows' milk when they reached the top of the milk ladder. Every child randomized, irrespective of allocation, received the same advice about progression on the ladder and the same support from a single investigator (Yd'A).

2.5 | Single low dose of whole milk at randomization

The previously validated ED_{05} of fresh milk (0.1 ml total volume, dilution as shown in Appendix 2) was chosen as the dose to be administered due to the low risk of reaction.[12] It was administered orally to every child in the intervention group by a single investigator (Yd'A), using a syringe, to avoid any topical contact on the face or lips. Blood pressure, pulse rate and oxygen saturations were measured before and at 15-minute intervals for 2 hours post-dosing. Criteria for a reaction to this single dose were as follows: any objective signs occurring within 2 hours of ingestion of the ED_{05}.[12,13,19,20]

2.6 | FAQL and STAI questionnaires

EuroPrevall Food Allergy related Quality of Life (FAQL) and parental expectation of outcome using the FAQL-Parent Form and Food Allergy Independent Measure (FAIM)[21] and State and Trait Anxiety Inventory (STAI) questionnaires were also completed.[22] State anxiety (S-Anxiety) reflects the psychological and physiological temporary reactions related to adverse situations when subjected to an anxiety-provoking stimulus. Trait anxiety (T-Anxiety) describes individual differences in the tendency to attend to, experience, and report negative emotions such as fears, worries and anxiety across many situations.[23] All measures are well-validated and used globally.

2.7 | Home implementation of baked milk and milk product introduction after intervention

After randomization, all parents were instructed in the use of the MAP milk ladder and requested to start it at home the day after randomization. Mothers were supported to continue breastfeeding according to their own preference. Skin prick test (SPT) was repeated at 6 and 12 months post-randomization, bloods were repeated at 6 months post-randomization and questionnaires completed at 1, 3, 6 and 12 months post-randomization. Follow-up was for 1 year from the date of randomization.

2.8 | Outcome measures

The primary outcome measure was level on the MAP milk ladder achieved by 6 months post-randomization. A progressor was defined as having reached an MAP milk ladder position of step 6 (of 12, lasagne) or above at 6 months, and a non-progressor was defined as having not reached step 6 by 6 months. Secondary outcomes were as follows: changes in food allergy-related quality of life (FAQL) measures from randomization to 1, 3, 6 and 12 months post-randomization, changes in maternal State and Trait Anxiety scores from randomization to 6 and 12 months and changes in milk SPT and serum levels of milk-specific IgE from 0 to 6 months in each group. We expected a significant decrease in State but not Trait Anxiety for intervention but not control group infants.

2.9 | Adverse events

A serious adverse reaction (SAE) was defined as requirement for emergency evaluation at hospital for any reason or parental administration of emergency medication such as adrenaline for any reason. Suspected unexpected serious adverse reactions (SUSAR) was defined as any adverse event relating to milk ladder progression that required medical evaluation or administration of emergency medication.

2.10 | Sample size and power calculation

To test a difference in proportions between intervention and control groups of 0.25 (a 25% difference in the rates of reaching the primary endpoint of step 6 at 6 months), 40 subjects were needed in the intervention group and 20 in the control group to find a change in probability of 0.0001 with a power of 0.8 and an alpha <0.05.[24] These hypotheses constitute a one-tailed test, justified by the observation in previous studies of no adverse effect/decreased tolerance of allergens after the single-dose intervention.

2.11 | Statistical analysis

The null hypothesis was that no difference exists in rate of completion of the MAP 12-step milk ladder, between those who consume the ED_{05} for milk under medical supervision at outset and those who do not consume the single dose of milk ED_{05} but receive the same follow-up care.

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 visit SPT wheal size and milk-specific IgE levels. Descriptive statistics were calculated for baseline scores on parent-report measures. Continuous variables were summarized as mean (with standard deviation) and categorical variables as frequency (percentage). A repeated measures analysis of variance was used to investigate change in scores over time for the whole group, irrespective of randomization. Analysis of covariance (ANCOVA) was used for testing the hypotheses about differences in means between the groups over time. Two-sided p-values were reported for all statistical tests; a p-value below 0.05 was considered to be statistically significant. Statistical analysis was performed using SPSS (version 26, IBM SPSS Statistics).

3 | RESULTS

3.1 | Cohort Description

A total of 109 outpatient referrals with possible milk allergy to established allergy centres were reviewed, and 78 attended for screening. Eighteen were designated screen fails: 17 had negative milk SPT and 1 had generalized urticaria. Sixty cow's milk allergic infants were recruited and randomized (Figure 2), 25 from CUH, 33 from CHI at Crumlin and 2 from OLOLD. Of these, 57 were followed up to 6 months and 55 to 12 months post-randomization (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. There was balanced randomization, with no significant difference in age, incidence of eczema, feeding method or time since last reaction between the intervention and control groups (Table 1). Egg sensitization, but not peanut sensitization, was significantly more common in the control group.

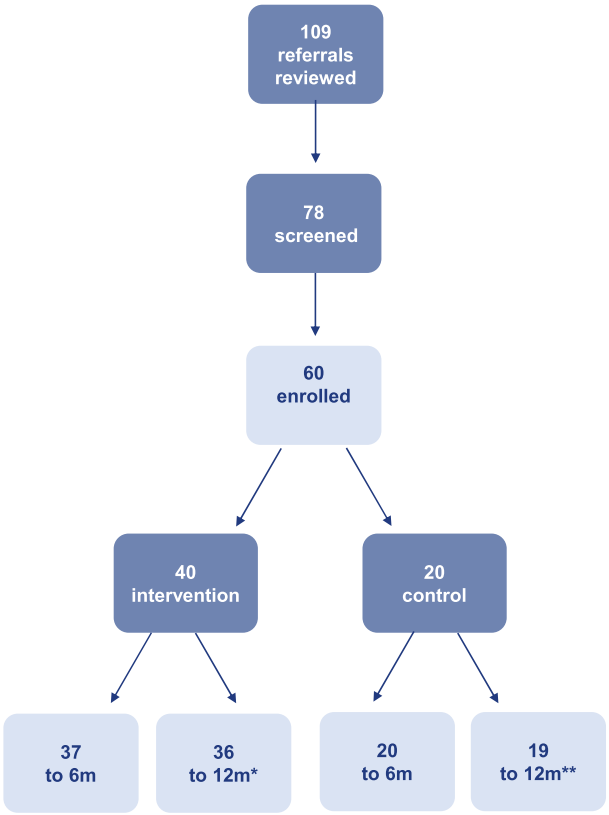


FIGURE 2 Study recruitment and subject flow

3.2 | Safety

There were 3 serious adverse events (SAEs) reported. One child attended an Emergency Department for acute viral laryngotracheobronchitis, unrelated to the study. Two children had reactions to milk at steps above their currently tolerated level on the milk ladder. One child had 2 accidental exposures in his out of home child care setting, one of which resulted in attendance at hospital and treatment with antihistamine. The other child was exposed to whole milk in a relative's house and was treated with antihistamine at home. There was no suspected unexpected serious adverse reaction (SUSAR) related to milk ladder progression.

TABLE 1 Subject demographics and baseline immunology data

	Intervention	Control	Total	*p*value intervention vs control
Sex	29/40 M	11/20 M	40/60 M	
Mean age (months)	7.3 (SD 0.38)	7.9 (SD 0.23)	7.5 (SD 2.1)	$p = .26$
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%)	$p = .68$
Egg sensitized	21/40 (53%)	19/20 (95%)	40/60 (67%)	$p < .001$
Peanut sensitized	19/40 (48%)	8/20 (40%)	27/60 (45%)	$p = .58$
Egg and peanut sensitized	12/40 (30%)	8/20 (40%)	20/60 (33%)	$p = .43$
Breastfed at recruitment	39/40 (98%)	20/20 (100%)	59/60 (98%)	
Days since last reaction (mean)	29.4 (SD 15.4)	31 (SD 12.2)	29.9 (SD 14.3)	$p = .67$

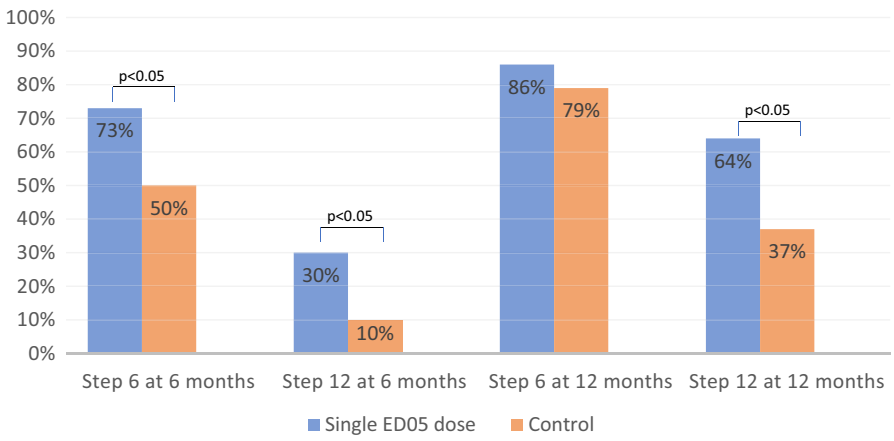


FIGURE 3 Milk Ladder Position at 6 and 12 months. Infants who received the single ED_{05} dose of milk at randomization were significantly more likely than control infants to have reached the primary end point (step 6 on MAP milk ladder) by 6 months and also to have completed the ladder by 6 months and at 12 months

3.3 | Single dosing

There were 4 reactors (10%) to the single ED_{05} of whole milk (Table S1). All four reactions were mild, with no treatment necessary in any case. Despite having reacted to the ED_{05} of milk, all 4 of these children progressed rapidly up the milk ladder: all 4 were on step 9 or above by 6 months, and all had reached step 12 (consuming whole milk without restriction) by 12 months post-randomization.

3.4 | Milk ladder position

At 6 months post-randomization, 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 ($p = .048$). Eleven of 37(30%) of the intervention group had already reached step 12 at 6 months compared with only 2/20 (10%) of the control group ($p = .049$, Figure 3). As a group overall, 37/57(65%) were on step 6 at 6 months, and 13/57(23%) were on step 12 at 6 months. This improved to 47/57(82%) on step 6 and 31/57(54%) at step 12, at 12 months. At 12 months post-randomization, 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, $p = .27$). However, significantly more of the intervention group 24/37(65%) had completed the ladder (step 12) compared to just 7/20(35%) of the control group (chi sq 4.7, $p = .03$), (Figure 3).

Baseline SPT was significantly associated with progressor status at 6 months (baseline SPT for progressors 5.7 mm vs 6.7 mm in non-progressors) but not 12 months (Figure 4). Baseline milk IgE was also significantly associated with progress or non-progress at 6 and 12 months (baseline milk IgE for progressors 4.7 KU_A/L vs 37.5 for non-progressors).

Progress on the milk ladder was significantly associated with changes in SPT, with a significantly larger decrease for progressors, irrespective of treatment group. This effect was greater in the treatment group: progressors in the treatment group, baseline SPT($M = 5.6$, SD = 1.6) and at 6 months SPT ($M = 2.3$, SD = 2.0); [$t(23) = -10.63$, $p = .0001$] and for progressors in the control group, ($M = 5.5$, SD = 1.4) and at 6 months ($M = 3.8$, SD = 2.2); [$t(9)$-3.43, $p = .008$).

3.5 | Maternal state and trait anxiety (STAI)

There was a statistically significant effect of time on State Anxiety (S-Anxiety), [$F = 4.85$, $p = .002$]. In contrast, maternal Trait Anxiety (T-Anxiety) scores did not change significantly over time [$F = 0.67$,

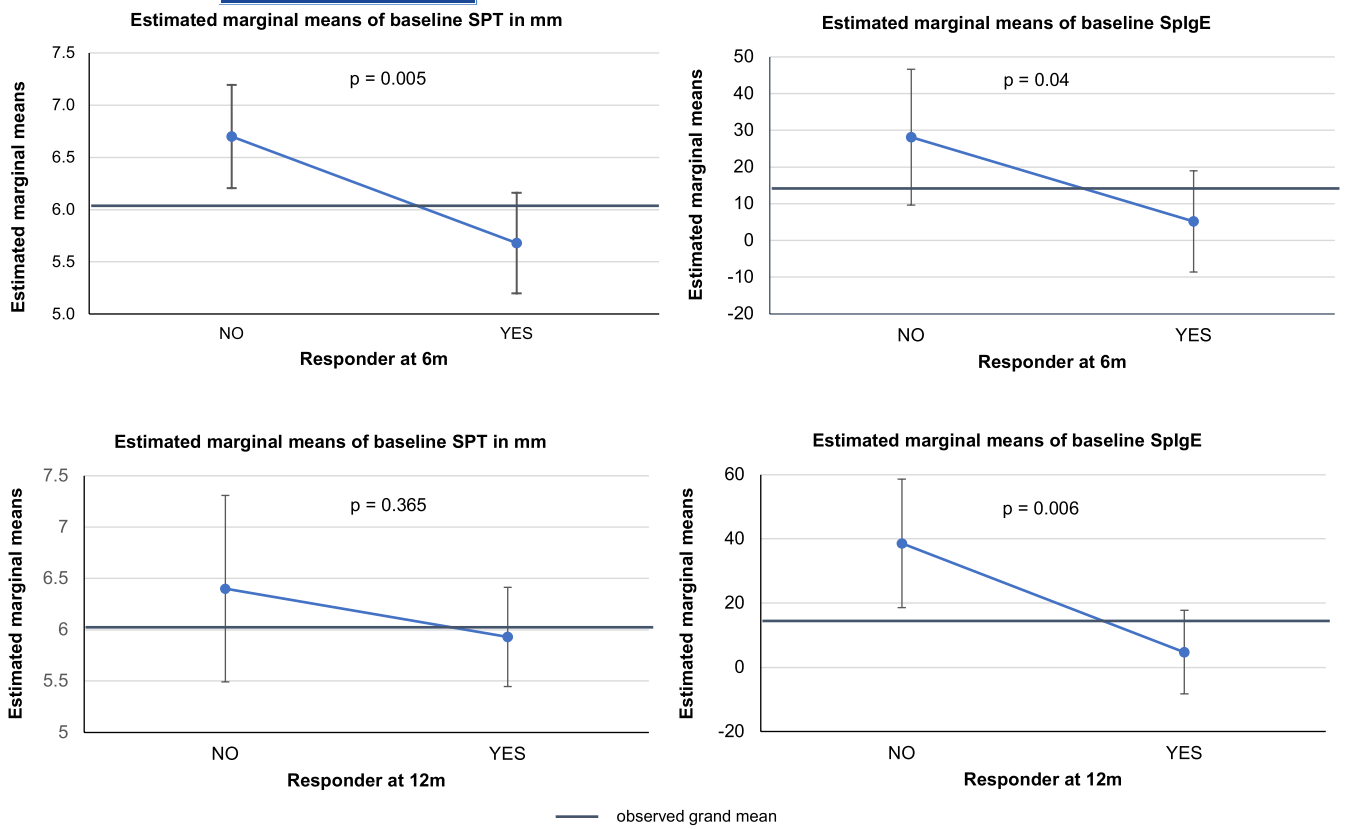


FIGURE 4 Baseline milk SPT and milk spIgE levels are associated with progressor status at 6 and 12 months. Infants who had reached the primary endpoint (MAP ladder step 6) by 6 months showed significant changes in SPT and spIgE at both 6 and 12 months

$p = .6$]. For the intervention group overall, there was a significant difference in maternal State Anxiety between scores at baseline ($M = 37.5$, SD = 12.9) and at 6 months ($M = 31.5$, SD = 8.6); [$t(32) = -2.81$, $p = .008$]. No significant difference was found in the control group overall for maternal State Anxiety for scores at baseline ($M = 33.1$, SD = 8.5) and at 6 months ($M = 31.7$, SD = 11.6); [$t(14)4.17$, $p = .59$).

3.6 | Effect of treatment outcome on maternal STAI

The groups were balanced at baseline, controlling for maternal State Anxiety and SPT in mm at baseline (main effects for group [$F(47) = 0.235$, $p = .63$). Scores on maternal State Anxiety at 6 months improved significantly from baseline for mothers of responders vs non-progressors (main effect for progress status [$F(47) = 4.751$, $p = .035$]). No significant difference in maternal Trait Anxiety was found in either the treatment group at baseline.

3.7 | Progressor/non-progressor status vs maternal State and Trait Anxiety

Progess on the MAP milk ladder after ED_{05} milk dosing had a marginal, non-significant impact on level of maternal Trait anxiety but State Anxiety decreased significantly for progressors in both treatment and control groups. For the treatment group, there was a significant difference in State Anxiety between scores at baseline (within group) for progressors vs non responders ($M = 37.9$, SD = 12.8) and at 6 months ($M = 33.2$, SD = 10.5); [$t(30) = -2.23$, $p = .03$]. 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, $p = .16$) These results suggest that progress with baked milk introduction using the MAP milk ladder has a significant impact on level of maternal State anxiety.

There was no correlation between baseline maternal STAI scores and baseline SPT or spIgE. However, maternal STAI scores were significantly associated with changes in SPT from baseline to 6 months and to 12 months (Table S2). This effect was more evident within a treatment group and when subjects were split according to being a progressor or non-responder (Table S3). Similar effects were seen for IgE (data not shown).

All progressors showed significant decreases in SPT; non-progressors showed no significant change. The 'high State Anxiety' non-progressors showed the least change. Findings were similar for Trait Anxiety at 6 m (data not shown). The findings relating to anxiety tertiles, changes in SPT in progressors and non-progressors were also consistent to 12 m (Table S4) with the only group to show an increase in SPT being the 'high State Anxiety' non-progressors.

3.8 | Food allergy quality of life and FAIM

FAQLPF and FAIM scores were similar in each group at baseline. FAQLPF score improved more than the minimum clinically important difference (MCID) in all but one group by 6 m and in all groups by 12 months. Changes in FAQLPF were statistically significantly different between progressors and non-progressors in the intervention group only (Table 2).

4 | DISCUSSION

We sought to compare the rate of ascent of the MAP milk ladder by cow's milk allergic infants, diagnosed between 6 and 12 months, between a cohort given a defined low-dose oral exposure to whole cow's milk immediately after diagnosis, and a control group. Both groups subsequently followed the identical plan for graded home introduction of milk containing foods, immediately starting a published milk ladder and receiving uniform support from a single investigator.

We showed a statistically significant difference between the 2 groups for the primary endpoint with a 23% absolute difference between the groups in getting halfway up the ladder (step 6) and also a 20% absolute difference in finishing the milk ladder/ drinking whole milk *ad libitum* (step 12). After 12 months, there was also a statistically significant difference between the 2 groups in how many infants had reached step 12 on the ladder. We think it is unlikely a once-only small dose of intact milk would lead to such a marked difference in rates of ladder completion by permanently accelerating progress towards immunological tolerance. We believe that collectively our data demonstrate that the very act of giving infants a single low dose of cow's milk in the presence of their mothers promoted parental confidence in home introduction, leading to accelerated progress up the milk ladder. Parents who have already experienced their child receiving whole milk—in the form of the single dose at recruitment—would have more confidence to progress to this last stage.

4.1 | Safety of single ED_{05} dosing in infancy

This study has reinforced data from previous studies of both older children with peanut allergy and similarly aged and older children milk allergy[12,13] showing single low-dose administration of an allergen is a safe procedure in infants, in this case at the time of first diagnosis of cow's milk allergy. However in this study, we did not use the single-dose challenge to establish which children were 'low-dose sensitive', rather to show parents in the intervention group their infant tolerated a small amount of whole milk, before they introduced baked milk at home. There were 4 reactors among the 40 intervention group children given the single ED_{05} dose. All 4 reactions occurred within a few minutes of administering the single dose, and all had resolved spontaneously without any treatment within 30 minutes. 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. Even though these 4 infants reacted to the single ED_{05} dose of milk, they all tolerated the baked milk products as listed at the bottom of the MAP ladder and all progressed rapidly up the milk ladder, all being 'progressors', past step 6 at 6 months. All 4 were also drinking whole milk by 12 months post-randomization. The safety of single exposure of infants to the ED_{05}, coupled with the positive influence of the intervention on movement up the MAP milk ladder indicates that this approach could be adapted to routine clinical practice in allergy services.

4.2 | Safety of milk ladder protocol in infancy

Previously published guidelines have recommended restricting home introduction of baked milk, to infants with mild cutaneous reactions only and those who have not experienced a reaction in the past 6 months ref.[11] In contrast, all 60 infants in this study were started on the MAP milk ladder at diagnosis, regardless of age or severity of initial reaction. 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, but no child was given adrenaline at any time during the study. There were only 3 non-tolerated accidental exposures to milk during the study. These data correlate with our data on home introduction of baked egg[25] and reinforce the safety data for home introduction of both milk and egg at diagnosis in the form of baked milk and egg.

4.3 | Outcome and efficacy of procedure

The intervention of the single ED_{05} dose immediately at diagnosis positively affects the outcome in CMPA infants using the MAP milk ladder and actually helps them to complete introduction of baked milk and then graded forms of milk at a faster rate.

TABLE 2 Food Allergy Quality of Life – Parent Form FAQL scores in progressors and non-progressors at baseline, 6 months and 12 months. Intervention group infants who reached the primary end point (MAP step 6 at 6 months—progressors—showed a significantly greater change in FAQLQ than non-progressors in the same group. Control group infants' FAQLQ did not change, irrespective of progress status

Treatment group	Progressor/non-progressor (P/NP)	FAQLQ baseline	FAQLQ 6 m	FAQLQ 12 m
Intervention	P	1.85	0.94*	0.53*
	NP	1.1	1.45	0.71
Control	P	1.51	0.8	0.85
	NP	2.2	1.1	0.97

Note: *For change from baseline value $p < .05$.

This study shows for the first time that maternal anxiety is associated with progress—or lack of progress—on the now widely used ladder approach to treating CMPA. Maternal Trait-anxiety levels are stable over time and are considered an important characteristic of patients with anxiety disorders, who present higher trait anxiety in comparison with healthy individuals.[23] In this study, maternal trait anxiety was inversely associated with milk ladder progress in both groups, with poorer outcomes in children whose mother had higher Trait-Anxiety levels.

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.

These findings relating to maternal anxiety have implications for the future management of cow's milk allergic infants and children with other food allergies. Clinicians can accurately identify the most anxious parents, who are most at risk of being unable to implement graded food introduction programmes. Clinicians could either use the supervised, single, low-dose approach demonstrated here as a simple way to provide reassurance or could put in place extra support to help these particular patients progress up the ladders. This could be either as simple as regular telephone support calls from the allergy team or more formal using group interventions such as cognitive behavioural therapy.[26]

FAQL and FAIM scores also tracked strongly with ladder progression. This is an expected outcome as, for infants of the age we studied, the FAQLQ-PF and FAIM measure parental perception of a child's quality of life and of the parent's expectation of outcome of future allergic events.[3,21] 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' and they may be more satisfied with the outcome of the treatment programme their child was offered.

4.4 | Limitations and strengths of this study

There are some limitations of this study. Firstly, there was no placebo group. We compared the intervention of the single dose with our current normal standard of care—active implementation of the milk ladder at diagnosis. Secondly, a formal double-blind placebo-controlled food challenge was not performed at the outset to confirm the suspected diagnosis. Such formal challenges are not considered essential in this age group and due to study design, children were seen extremely quickly after referral and had typical histories and supportive IgE-based tests. Such challenges may have biased parental engagement with milk ladder use by demonstrating tolerability of low doses of milk. Thirdly, all the referred cases of possible CMPA were mild or moderate in severity. This may be due to the very short time from referral to assessment according to study protocol and due to very limited access to allergy services in Ireland, biased referral elsewhere of more severe cases can be discounted. There was unselected, rigorous screening of participants, and recruitment was based on standard clinical criteria already shown to be effective.[11,12,19] Fourthly, this study was not blinded to treatment allocation; however, a single researcher completed all appointments leading to a uniformity in all study based procedures such as SPT, administration of questionnaires and advice given on use of the milk ladder by both groups during the course of the study. A formal exit challenge with milk, after its exclusion for a few weeks, was not performed so the establishment of immunological tolerance was not formally assessed. Safe regular consumption of formula milk or whole cow's milk (step 12 of the MAP) ladder would suggest the children are at least desensitized and many would be expected to be fully tolerant.

5 | CONCLUSIONS

Use of a milk ladder or a similar support document and therapeutic support are safe even in infants with cow's milk allergy (CMPA), including those who have had anaphylaxis before referral or who react to the milk ED_{05} during a supervised feed. Furthermore, no delay between diagnosis and introduction of baked milk is required. A supervised single dose accelerates progression with ladder-based introduction of baked milk and dairy products. This is most likely due to giving the parents the confidence to proceed. Wider adoption of early, rather than delayed use of the milk ladder, supported by the use of the validated single-dose practice shown here, could lead to much earlier clinical resolution of CMPA, with all of the nutritional and social advantages associated with early resolution. Maternal anxiety must be taken into consideration when assessing treatment plans for food-allergic children, who can be assisted to become tolerant of milk much more quickly than had been previously reported, and without multiple hospital attendances.

ACKNOWLEDGEMENTS

We thank the families of the babies involved, the staff of each hospital's clinical research centre and specifically Alicia Wallace RN and Marc O'Sullivan PhD for clinical and technical assistance. Open access funding provided by IReL.

CONFLICT OF INTEREST

AB received research funding from Aimmune Therapeutics and speaker fees from Nutricia, RvR declares consultancies for HAL Allergy BV, Citeq BV, Angany Inc., speaker fees from HAL Allergy BV, ThermoFisher Scientific, ALK and stock options from Angany Inc. ADG receives research funding and speaker fees from Aimmune Therapeutics and DBV Technologies, JO'BH receives research funding, speaker fees and consultancy fees from Aimmune Therapeutics, research funding and speaker fees from DBV Technologies, research funding from Johnson&Johnson, Clemens von Pirquet Foundation and Temple St Hospital Research Foundation.

AUTHOR CONTRIBUTIONS

YMd'A and JO'BH involved in conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, visualization, writing–original draft, and writing–review and editing. LF involved in data curation, formal analysis, writing–original draft, and writing–review and editing. AMB and JF involved in conceptualization, formal analysis, investigation, methodology, visualization, and writing–review and editing. RvR and ADG involved in conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing–original draft, and writing–review and editing.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: d'Art YM, Forristal L, Byrne AM, et al. Single low-dose exposure to cow's milk at diagnosis accelerates cow's milk allergic infants' progress on a milk ladder programme. *Allergy*. 2022;77:2760–2769. doi:10.1111/all.15312