





Single-dose oral challenges to validate eliciting doses in children with cow's milk allergy

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Abstract

Background: There is increasing interest in the use of eliciting doses (EDs) to inform allergen risk management. The ED can be estimated from the distribution of threshold doses for allergic subjects undergoing food challenges within a specified population. Estimated ED₀₅ values for cow's milk (the dose expected to cause objective allergic symptoms in 5% of the milk-allergic population) range from 0.5 mg to 13.9 mg cow's milk protein. We undertook a single-dose challenge study to validate a predicted ED₀₅ for cow's milk of 0.5 mg protein.

Methods: Participants were recruited from 4 clinical centres. Predetermined criteria were used to identify patients reacting to 0.5 mg cow's milk protein (approximately 0.015 mL of fresh cow's milk). Children over 1 year underwent formal challenge to cow's milk to confirm clinical reactivity.

Results: 172 children (median age 6.0 (IQR 0.7-11) years, 57% male) were included in this analysis. Twelve (7.0%, 95% CI 3.7%-11.9%) children experienced objective symptoms that met the predetermined criteria. One participant had mild anaphylaxis

Abbreviations: CMPA, Cow's milk protein allergy; DBPCFC, Double-blind placebo-controlled food challenge; ED, Eliciting dose; IQR, Interquartile range; OFC, Oral food challenge; VITAL, Voluntary Incidental Trace Allergen Labelling.

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that responded to a single dose of adrenaline, the remainder experienced only mild symptoms with no treatment required. We did not identify any baseline predictors of sensitization that were associated with objective reactivity to the single-dose challenge using 0.5 mg cow's milk protein.

Conclusions: These data support an estimated ED₀₅ for cow's milk of 0.5 mg protein. Values for ED₀₅ above 0.5 mg for cow's milk protein proposed for allergen risk management need to be reviewed.

KEYWORDS

cow's milk, eliciting dose, single-dose challenge, thresholds, Voluntary Incidental Trace Allergen Labelling (VITAL)

Key Message

In this multicentre study, we validated the eliciting dose (ED) for cow's milk at around 0.5 mg cow's milk protein (ED₀₅), and thus, higher estimates currently proposed to inform allergen risk management should be reviewed.

1 | INTRODUCTION

There is increasing interest in the use of routinely collected clinical data from oral food challenges (OFC) to inform both patient management and allergen risk management in industry, in terms of the level of dietary allergen avoidance required. Eliciting doses (ED) for allergic reactions in 1% and/or 5% of the allergic population (ED₀₁ and ED₀₅, respectively) can be used to inform 'reference doses', indicating a level of allergen presence above which additional risk management strategies (such as precautionary allergen labelling) are required to protect the allergic population.^{1,2} In addition, it has been proposed that dietary advice to food-allergic consumers might be individualized if a particular level of tolerance can be demonstrated at clinical OFC.^{3,4}

ED values are generated from OFC data,⁵ but many OFC protocols use a starting dose that will trigger symptoms in a significant proportion of patients. For example, the PRACTALL consensus recommends a starting dose of 3 mg food protein for OFC,⁶ but data suggest that for cow's milk protein allergy (CMPA), this may cause objective symptoms in 10% of allergic individuals.⁷ Thus, these data are 'left-censored' and cause greater uncertainty when estimating a level of exposure, which causes symptoms in a small proportion of the allergic population.⁵ Conventional protocols, which use incremental doses given every 20–30 minutes also make it difficult to reliably determine the precise dose that has triggered symptoms, but this uncertainty can be reduced by using both discrete and cumulative doses to estimate ED values.⁸ In addition, relying on OFC undertaken in routine clinical practice can result in selection bias, since subjects at high likelihood of true clinical reactivity or with a history of prior anaphylaxis may be excluded.⁶

CMPA is a major cause of severe and even fatal allergic reactions.^{9,10} Data from the United Kingdom have found that cow's milk is the confirmed trigger in over a quarter of anaphylaxis

fatalities in children,¹¹ a pattern that has also been noted in North America and Israel.^{12–14} This is probably due to a combination of factors: milk as an ingredient that is ubiquitous in our diets; milk as a high protein food; and lower levels of awareness amongst the public and food business operators that CMPA can cause severe reactions.¹⁵ Reported estimated ED₀₅ values for cow's milk in the literature range from 0.21 mg to 13.9 mg cow's milk protein.^{2,7,16,17} This variation may be partly explained by differences in patient selection, with some studies including younger patients (many of whom may be at various stages of outgrowing their milk allergy) or with lower levels of sensitization, which has been associated with higher levels of reactivity at challenge.¹⁷ We have previously used a novel, single-dose challenge design to validate the ED₀₅ for peanut.³ In this study, we sought to replicate this method in children with cow's milk protein allergy (CMPA), to assess whether current estimates for ED₀₅ for cow's milk are valid in terms of allergen risk management.

2 | METHODS

This was a multicentre study that incorporated children with CMPA recruited from 4 clinical centres: Imperial College London—St Mary's Hospital, UK (Imperial); Hospital Clinico San Carlos (HCSC) and Hospital Universitario Infantil Niño Jesús (NIJH) in Madrid, Spain; and Cork University Hospital, Ireland (CUH); the specific cohorts are described in Table 1. Exclusion criteria were as follows: medically unfit for challenge according to local unit OFC guidelines/protocol (eg high fever or unwell with intercurrent illness); acute wheeze or poorly controlled asthma symptoms (as defined by clinician judgement with reference to the ICON consensus¹⁸) or oral corticosteroids within 14 days of

TABLE 1 Characteristics of included cohorts

Centre	Ireland	Madrid, Spain		United Kingdom
	Cork University Hospital (CUH)	Hospital Clinico San Carlos (HCSC)	Hospital Universitario Infantil Niño Jesús (NJH)	Imperial College London (Imperial)
Inclusion criteria	History of unequivocal exposure (including accidental) and typical acute allergic reaction within the preceding 2 mo and evidence of IgE sensitization (SPT or sIgE) to cow's milk. OR Positive OFC to cow's milk within 2 mo of the single-dose challenge.		History consistent with IgE-mediated allergy to CM and Positive DBPCFC to cow's milk immediately following single-dose challenge.	
Inclusion age:	0-16 y	Any	6-17 y	
Challenge formulation:	>1 y: Milk powder incorporated into a chocolate dessert matrix <1 y: Fresh cow's milk	Milk powder incorporated into a chocolate dessert matrix	Milk powder dissolved in rice "milk" as part of a DBPCFC	
Blinding for single-dose challenge	Open	Open	Double-blind	
Observation period:	2 h	2 h	Minimum 1 h post-dose, with no objective symptoms within 2 h	
Clinical reactivity confirmed by:	>1 y: open OFC <1 y: allergic reaction within 2m of assessment and IgE sensitization	Objective symptoms at oral exposure to cow's milk (eg OFC, DBPCFC) under medical supervision	Objective symptoms at DBPCFC	

OFC; anaphylaxis of any cause in the 4 weeks prior to OFC (to exclude patients in an anergic state); and antihistamines within 5 days of OFC. In order to minimize selection bias, routine clinic lists were screened for patients with IgE-mediated allergy to cow's milk, and then, participation discussed with all potentially suitable participants and their families during routine clinic appointments. Subjects with a history of prior anaphylaxis were not excluded. The studies were registered at Clinicaltrials.gov (NCT02216175, NCT02295397).

2.1 | Food challenge

Protocols were aligned across the 4 centres in order to obtain the same clinical data following 0.5 mg cow's milk protein (approximately 0.015 mL of fresh cow's milk) administered as a single dose, using the same predefined case definition for objective allergic symptoms. In general, the single-dose challenge was administered as milk powder incorporated into an allergen-free chocolate dessert matrix (previously validated for double-blind challenges¹⁹) or dissolved into flavoured rice 'milk' (Table 1). In participants under age 1 year at CUH, the dose was instead administered as diluted (1:7) fresh milk using a syringe (to reduce the risk of a contact reaction to the lips). Routine OFC monitoring was undertaken according to local practice. At two centres (Imperial and NJH), formal DBPCFC were conducted, where the 0.5 mg dose constituted the first dose of the OFC; subjects were observed for at least 1 hour prior to the next challenge dose being administered (and longer if there were any non-transient symptoms). At HCSC and CUH, subjects underwent a single (unblinded) administration of 0.5 mg protein and were observed for at least 2 hours thereafter.

2.2 | Criteria for a positive OFC result and case definition

Data collection and case definitions have been previously described.³ In brief, detailed notes were taken recording all physical or behavioural changes observed or self-reported during the single-dose OFC. Predetermined objective criteria were used, since published ED₀₅ values are predicted on the basis of challenge-associated objective symptoms only.¹⁻⁶ The predetermined objective criteria for a positive single-dose OFC result were as follows: 3 or more concurrent wheals of non-contact urticaria persisting for at least 5 minutes; perioral or periorbital angioedema; rhinoconjunctivitis (including sneezing) for at least 5 minutes; diarrhoea; vomiting (excluding gag reflex); or anaphylaxis (with evidence of circulatory or respiratory compromise, such as persistent cough, wheeze, change in voice, stridor, difficulty breathing, and collapse).²⁰ Transient objective symptoms (rhinoconjunctivitis <5 minutes, transient mild erythema) were excluded. Subjective symptoms were also recorded. Following completion of the clinical studies, cases were reviewed by at least 2 senior independent investigators and the above criteria were applied to define OFC which met these predetermined objective criteria.

2.3 | Confirmation of clinical reactivity to cow's milk

In order to avoid the possibility of including participants without CMPA, clinical reactivity was confirmed in participants over 1 year of age at formal oral exposure, typically double-blind placebo-controlled challenge conducted according to international

PRACTALL consensus criteria,⁶ although some families declined DBPCFC and instead underwent an unblinded challenge under medical supervision, which required objective symptoms to be assigned as 'positive'. Infants (under 12 months) did not undergo OFC, but were included on the basis of physician-diagnosed allergic reaction within 2 months of assessment and IgE sensitization to milk.

2.4 | IgE sensitization

Blood samples were collected from participants prior to OFC. Samples were processed according to the manufacturers' instructions and snap-frozen at -80°C until analysis. Specific IgE to cow's milk and casein was measured using ImmunoCAP (Thermo Fisher Scientific). Skin prick testing was undertaken according to international guidelines using ALK lancets and commercial extracts (ALK-Abello) with 1% histamine as a positive control, and the mean wheal diameter noted.

2.5 | Statistical analyses

Analyses were planned prospectively. The proportion of participants reacting to 0.5 mg cow's milk protein was estimated with 2-sided exact 95% confidence intervals. Baseline characteristics across cohorts were compared using Kruskal-Wallis test since the data were not normally distributed. Receiver operating characteristic (ROC) curves were generated in order to identify possible predictors for reactivity to 0.5 mg cow's milk protein. A P value of $< .05$ was considered significant. Assuming a reaction rate of 5% to 0.5 mg cow's milk protein, an overall sample size of 150 and 250 would correspond to a lower 95% confidence limit of 2.1% and 2.8%, respectively, and an upper confidence limit of 9.8% and 8.7%, respectively, for the estimated ED_{05} .

2.6 | Ethical approval

Local approvals were obtained for each clinical centre: Imperial, NHS Human Research Authority reference 15/LO/0286; HCSC, Ethics Committee reference 14/345; NJH, Ethics Committee reference R0003/17; CUH, reference ECM4(N) 03/06/14 and ECM4(U) 04/07/17. Written informed consent was obtained from all participants or their legal guardian, and patient assent was obtained where appropriate.

3 | RESULTS

A total of 267 children were screened for inclusion between August 2015 and September 2020, of whom 182 underwent OFC to 0.5 mg

cow's milk protein. Ten individuals went on to pass a formal food challenge (ie did not react to a minimum of 250 mL cow's milk) following the 0.5 mg challenge and were therefore excluded from the primary analysis. Baseline demographics are shown in Table 2. The clinical centre in Ireland predominantly recruited children under age 1 year with CMPA, HCSC recruited infants with new diagnosis of CMPA as well as patients over age 1 year with an existing diagnosis of CMPA, while other centres recruited children with persistent CMPA. Overall, 61 (34%) of the cohort were under age 1 year (recruited at CUH and HCSC); participants at NJH and Imperial were older ($P < .001$, Kruskal-Wallis test). IgE sensitization was similar across all 4 cohorts in terms of skin prick test wheal, but serum IgE to cow's milk was lower in the CUH cohort ($P = .04$), but equivalent across the other 3 cohorts ($P = .10$), reflecting the lower age of the included participants.

Clinical reactivity was confirmed at OFC in 69% of participants (and 99% of participants older than 1 year of age). Of these OFC, 84% were DBPCFC conducted according to PRACTALL consensus. The family of an 8-year-old male in the HCSC cohort with a history of multiple anaphylaxis events to milk (including bronchospasm to a small piece of chocolate 1 month prior to the single-dose challenge) declined OFC, but the child was enrolled in a local oral immunotherapy programme and experience objective symptoms (generalized urticaria and bronchospasm during up dosing), thus confirming clinical reactivity. Eliciting dose at formal OFC to cow's milk in each cohort is shown in Table 2, and the dose distribution is summarized in Figure 1. There were no differences across the cohorts in terms of eliciting dose ($P = .29$), implying that the 4 cohorts were similar to each other in terms of clinical reactivity. We did not observe any correlation between age and eliciting dose at formal challenge (Spearman's $r = .05$, $P = .59$).

3.1 | Reactions to challenge using 0.5 mg cow's milk protein

Of the 172 OFC eligible for inclusion, 122 (71%) showed no symptoms (Table 3). A total of 33 (19%) participants reported transient subjective symptoms, while 17 had objective symptoms, of which 12 (7.0%, 95% CI 3.7%–11.9%) met the predetermined challenge-positive criteria. These reactions are documented in Table 4. One participant, a 17-year-old, experienced mild chest tightness that was associated with bilateral wheeze on auscultation and a 25% drop in peak expiratory flow rate, and mild truncal erythema; these symptoms responded to a single dose of intramuscular adrenaline. Otherwise, reactions were mild and did not require treatment. There was no difference in the rate of positive reactions to 0.5 mg protein by challenge matrix formulation ($P = .42$, Fisher's exact test) or challenge design (single-dose challenge vs DBPCFC, $P = .24$, Fisher's exact test). We did not identify any predictors of reactivity to 0.5 mg cow's milk protein using ROC curve analysis (Table 5).

TABLE 2 Baseline demographics of participants who underwent single-dose challenge to cow's milk

Centre	Ireland	Madrid, Spain		UK	Overall
	CUH	HCSC	NJH	Imperial	
Screened	Age <1 y: 65 >1 y: 11	60	64	67	267
Did not meet inclusion criteria for OFC or refused to participate	13	30	33	9	85
Underwent single-dose challenge	Age < 1 y: 57 >1 y: 6	30	31	58	182
Age (median, IQR)	0.6 y (0.5-0.7)	5 y (2-6)	9 y (8-12)	11 y (8-14)	6 y (0.7-11)
Sex (%male)	63%	42%	52%	64%	57%
History of prior anaphylaxis ^a to cow's milk	6/62 (10%)	8/24 (33%)	17/31 (55%)	29/58 (52%)	60/182 (33%)
Excluded due to tolerance to CMPA at subsequent OFC	1	6	1	2	10
Total "valid" single-dose challenges	62	24	30	56	172
Inclusion criteria					
Positive OFC	11/62 (18%)	21/24 (88%)	30/30 (100%)	56/56 (100%)	118/172 (69%)
Reaction last 2m	51/62 (82%)	3/24 (13%)	n/a	n/a	54/172 (31%)
Serum IgE to:					
Cow's Milk (median, IQR)	3.9 (1.2-15.6)	10.8 (1.7-27.6)	20.5 (6.8-87.4)	19.9 (3.0-56.4)	10.3 (2.1-43.9)
Casein (median, IQR)	1.0 (0.2-8.3)	2.7 (0.35-21.0)	13.0 (2.7-81.1)	14.2 (2.6-52.0)	6.4 (0.8-27.4)
Skin Prick test (mm):					
Cow's Milk (median, IQR)	7 (5-9)	6 (5-7)	7 (5-8)	7 (5-9)	7 (5-8)
Casein (median, IQR)	n/a ^b	5 (3-8)	6 (5-9)	7 (5-9)	6 (4-9)
SPT ≥8 mm (or 6mm for patients under 2 y) ²⁹ OR sIgE ≥15 kUA/l ³⁰	43 (69%)	13 (62%)	21 (70%)	42 (75%)	119 (70%)
Eliciting dose at formal OFC (mg protein)					
Median	170	1433	444	144	433
IQR (number)	(68-340) (n = 11)	(228-1659) (n = 21)	(44-4444) (n = 30)	(44-1444) (n = 56)	(76-1659) (n = 118)

^aAnaphylaxis defined as prior cardiovascular or lower respiratory compromise.

^bn/a : casein skin test extract not available in Ireland.

These data therefore broadly validate the estimated ED₀₅ for cow's milk of 0.5 mg protein (with potential reactions occurring in an interval between 3.7% and 11.9% of the milk-allergic population).

4 | DISCUSSION

Single-dose OFC has previously been used to validate the estimated ED₀₅ for peanut, derived from statistical dose-distribution modelling of individual patient threshold doses.³ In this study, we utilized a similar approach to validate proposed ED₀₅ estimates for cow's milk. The observed proportion of patients reacting to 0.5 mg cow's milk protein (approximately 0.015 mL of fresh cow's milk) with

predetermined objective criteria was 7.0% (95% CI 3.7%-11.9%). This is within the statistical bounds for the original estimated ED₀₅ of 0.5 mg cow's milk protein that would result in 5% of the milk-allergic population reacting with objective symptoms. These data therefore imply that proposed ED₀₅ values greater than 0.5 mg over-estimate the true ED₀₅ for cow's milk.

The use of population EDs has been proposed by the food industry to establish action levels above which measures are required for risk management, such as the use of precautionary allergen labelling.²¹ One such scheme is the Voluntary Incidental Trace Allergen Labelling (VITAL) in Australia. The VITAL Scientific Expert Panel recently updated reference doses for major food allergens, using updated OFC datasets and a new Stacked Model Averaging algorithm

incorporating five different statistical models (Weibull, Log Logistic, Log Normal, Log Double Exponential, General Pareto).^{2,22} For cow's milk protein, an ED₀₅ of 2.4 mg (95%CI 1.3 to 5.0) was proposed, although the action level was based on an ED₀₁ of 0.2 mg (95%CI 0.1 to 0.5). Prior to the updated VITAL publication, estimated ED₀₅ values for cow's milk derived from the analysis of multiple cohorts varied from 0.57 mg to 1.9 mg. This variation is mainly due to the uncertainty resulting from a lack of data with respect to low-dose reactors, a phenomenon that particularly affects cow's milk OFC.² In the latest analysis by the VITAL Scientific Expert Panel, over 21% of data was left-censored (ie patients with CMPA reacted to the first OFC dose) and 75% of included data were derived from OFC where the initial dose was >1.5 mg protein (and often significantly more so).² In addition, current estimates rely on data from routine clinical challenges where subjects may be excluded (eg due to prior anaphylaxis or recent reaction) and so the resulting dose-distribution

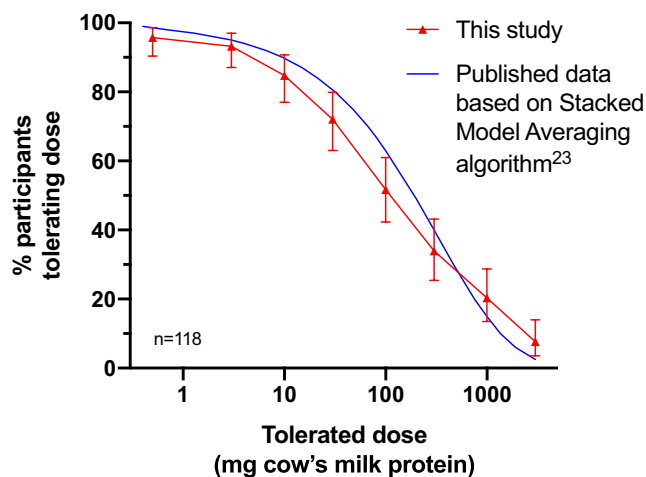


FIGURE 1 Threshold dose distribution for the 118 participants who also underwent formal food challenge to cow's milk, compared to published dose-distribution data by Houben et al²² Error bars represent 95% confidence intervals

curves may not represent the true allergic population. Importantly, heterogeneity in patients with cow's milk allergy is much more likely to be a confounder compared to other food allergies. This is because many included subjects may be in a transition towards natural resolution; there is evidence that this dynamic nature of milk allergy in younger children will impact on reaction threshold levels at food challenge.¹⁷ These are the pivotal justifications for single-dose challenges (such as this study) to validate the estimated ED at the lower end of the dose distribution curve where data have been lacking, using a patient cohort that includes subjects who are less likely to outgrow their milk allergy in early childhood.

It is particularly important to have certainty over EDs used for allergen risk management in CMPA. Cow's milk is increasingly ubiquitous in our diets; its protein fractions are soluble and both (liquid) milk and milk powder tend to distribute well in formulations resulting in a homogenous distribution throughout a food product (as opposed to particulate distribution associated with allergens such as nuts).^{9,23} It is a frequent cause of severe and even fatal allergic reactions⁹⁻¹⁴ and can be difficult to eliminate from food production lines (eg those used to produce chocolate-based products) to the extent that a significant proportion of dark chocolate products (made without cow's milk as an ingredient) contain significant levels of cow's milk protein due to shared production.^{23,24} In validating the ED₀₅ for cow's milk as 0.5 mg protein, these data indicate that current estimates for ED₀₅ for cow's milk based on population modelling using existing data are too high. Additional, larger challenge data sets (based on dosing schedules that would allow for interval censoring) are needed to provide more precision to the population dose-distribution modelling around lower ED values.

These data are also relevant to the selection of appropriate protocols for clinical challenges to diagnosis CMPA. In general, the initial doses recommended for DBPCFC under the PRACTALL consensus are 3 mg protein,⁶ which for most allergens will tend to cause objective symptoms in around 10% of individuals (ED₁₀).^{1,7} If the ED₀₅ for cow's milk is closer to 0.5 mg, then well over 10% of individuals with CMPA would be expected to react to an initial dose of 3 mg.

	Ireland	Madrid, Spain		UK	Overall
Centre	CUH	HCSC	NJH	Imperial	
Eligible participants (completed OFC)	62	24	30	56	172
Outcome:					
No symptoms	54	22	18	28	122
Transient subjective symptoms only	n/a ^b	0	10	23	33
Any objective symptoms	8	2	2	5	17
Objective symptoms ^a	8	0	1	3	12
Anaphylaxis	0	0	0	1	1

^aObjective symptoms which met predefined criteria.

^bDue to participant age, it was not possible to observe study-defined subjective symptoms in the majority of participants at CUH.

TABLE 3 Symptoms experienced to single-dose challenge to cow's milk

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

ID	Centre	Age (y)	Sex	Inclusion	SPT to CM extract (mm)	slgE to CM (kUA/l)	Time to symptoms	Challenge Symptoms
Ui-14	CUH	0.9	F	Recent reaction in last 2m and sensitised	5	15.4	<5 mins	Vomiting
Ui-40	CUH	1.3	F	Recent reaction in last 2m and sensitized	4	3.2	15-20 mins	Urticaria, lip angioedema, eczema flare
Ui-66	CUH	2.6	M	Recent reaction in last 2m and sensitized	5	0.95	<5 mins	Periorbital angioedema, abdominal pain, eczema flare
Ui-72	CUH	0.2	F	Positive formal OFC	3	7.8	<5 mins	Vomiting
U1-26	CUH	0.5	M	Recent reaction in last 2m and sensitized	6	1.36	5-10 mins	Lip angioedema, urticaria
U1-29	CUH	0.9	M	Recent reaction in last 2m and sensitized	5	4.42	5-10 mins	Urticaria
U1-36	CUH	0.7	M	Recent reaction in last 2m and sensitized	6	ND	5-10 mins	Urticaria
U1-50	CUH	0.4	M	Recent reaction and sensitized	5	1.48	5-10 mins	Urticaria
S101	Imperial	17	F	Positive DBPCFC	12	29.6	<5 mins	Bilateral wheeze, erythema
S129	Imperial	14	F	Positive DBPCFC	13	>100	38 mins	Persistent rhinoconjunctivitis
S155	Imperial	10	F	Positive DBPCFC	11	80.4	24 mins	Lip angioedema, oropharyngeal pruritus
S214	NJH	8	M	Positive DBPCFC	9	83.4	15 mins	Persistent dry cough, vocal hoarseness

Abbreviation: ND, not done due to insufficient blood sample.

TABLE 5 Predictors of reactivity to single-dose challenge of 0.5 mg cow's milk protein

Biomarker	Area under ROC curve	P value
slgE to cow's milk	0.50	.98
slgE to casein	0.57	.56
SPT to cow's milk extract	0.55	.57
SPT to casein	0.54	.76

Furthermore, many challenge protocols used in clinical practice start with higher doses of 1 mL cow's milk (approximately 30 mg protein),^{25,26} to which around 25% of allergic individuals will react. In the context of OFC where patients may have a higher likelihood of clinical reactivity (eg prior to commencing allergen immunotherapy), clinicians might therefore wish to choose a lower initial challenge dose to which objective symptoms are unlikely (eg to build confidence in the patient and their family).

4.1 | Strengths and limitations of this study

The international collaboration, robust protocol and the use of predetermined objective, challenge-positive criteria to demonstrate

true clinical reactivity (including by OFC in 67%, of which 84% were DBPCFC) are strengths of this study. Infants in one of the Cork cohorts underwent challenges using liquid milk rather than milk powder; however, the estimated EDs for liquid milk and milk powder are equivalent.⁷ We chose to recruit a significant proportion of participants under 1 year of age, since CMPA is more prevalent in this age group, but also included teenagers with persistent CMPA who are often excluded from challenge studies. It is possible that very young children with CMPA are more likely to outgrow this allergy and this may be reflected in eliciting doses at challenge. However, we did not identify any differences across the included cohorts in terms of eliciting dose, although the number of very young children in this subanalysis was limited.

To minimize selection bias, we utilized a recruitment strategy that did not involve the subjective selection of participants by healthcare professionals, nor did we exclude participants with a history of anaphylaxis. Furthermore, the distribution of eliciting doses at challenge in this study (as depicted in Figure 1) is not dissimilar to published data for cow's milk,²² which strongly supports our assessment that our participants are very likely to represent the population with CMPA in Europe. We were unable to determine the proportion of subjects with tolerance to baked milk, as this had not been assessed in the majority of subjects. In any event, data suggest that eliciting doses in individuals with or without tolerance to the baked allergen

do not differ significantly.²⁷ While there are some very limited data to indicate that adults with CMPA may have a higher threshold than children (on the basis of OFC data from 25 adults and 323 children),¹ we did not identify an age-dependent effect amongst the participants recruited in this study.

Just over half of the single-dose OFC were undertaken using a double-blind methodology, with the 0.5 mg dose constituting the first dose at DBPCFC (with prolonged observation interval prior to the 2nd dose being administered). We did not observe a significant difference in frequency of objective reaction to 0.5 mg cow's milk protein between those who underwent an open challenge and those who had DBPCFC. Two subjects who underwent DBPCFC reacted with objective symptoms in the 2 hours following the 0.5 mg dose: one had no symptoms whatsoever to the 0.5 mg dose, but rapidly developed objective symptoms to the next dose administered; the second had subjective symptoms that completely resolved for at least 60 minutes prior to the next dose administered. Given these observations and published data that reactions to cow's milk occur more rapidly than for other allergens,²⁸ it is very unlikely that the reactions in these 2 participants were due to the 0.5 mg dose. Finally, these data apply to challenge conditions, where allergic individuals are clinically well and usually without the presence of co-factors, which might impact on reaction thresholds. Our aim in this study was not to assess the risk of participants reacting to 0.5 mg doses due to the presence of co-factors.

5 | CONCLUSIONS

In summary, we have demonstrated that the ED₀₅ for cow's milk is likely to be around 0.5 mg protein and certainly lower than some of the proposed values for ED₀₅ in the literature. These data demonstrate the need to validate estimated ED values derived from dose-distribution analyses of data in studies not limited by left censoring, in order to identify the most highly dose-sensitive population of patients with food allergy. This will assist regulators, public health agencies and food business operators in establishing evidence-based approaches to allergen management as means to protect the food-allergic consumer from accidental exposures.

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CONFLICT OF INTEREST

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