Reproducibility of food challenge to cow's milk: Systematic review with individual participant data meta-analysis

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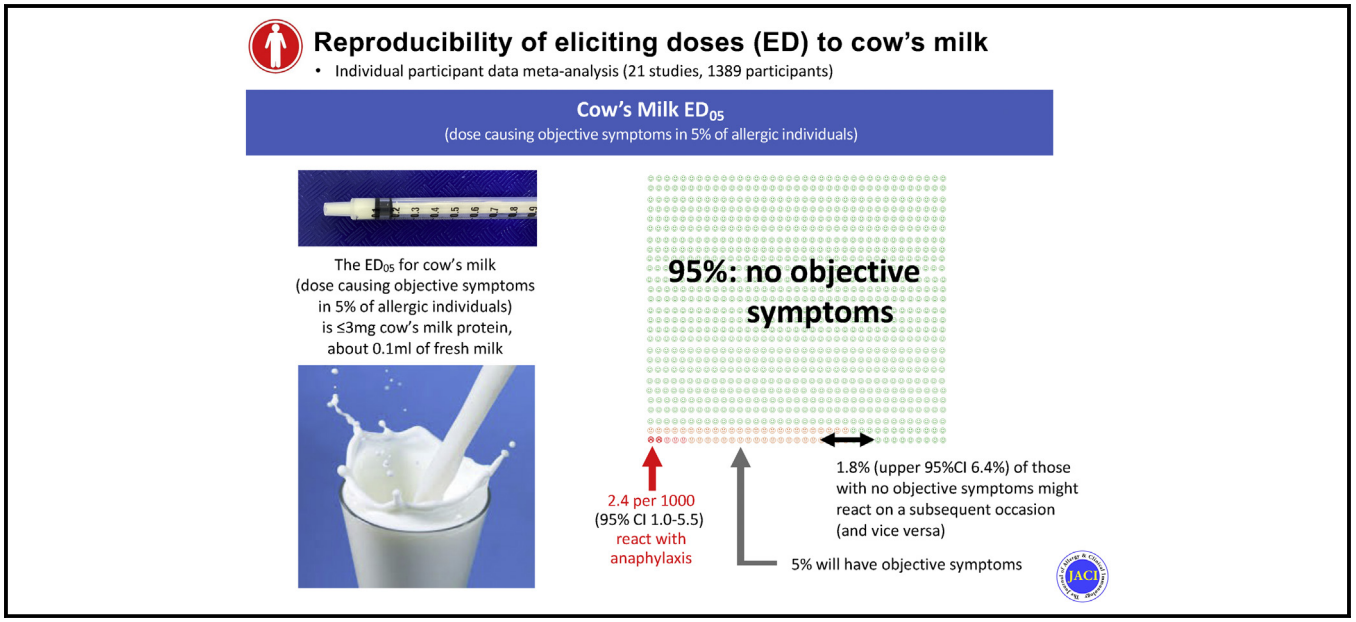
GRAPHICAL ABSTRACT



Background: Cow's milk (CM) is an increasingly common cause of severe allergic reactions, but there is uncertainty with respect to severity of reactions at low-level CM exposure, as well as the reproducibility of reaction thresholds.

Objective: We undertook an individual participant data (IPD) meta-analysis of studies reporting double-blind, placebo-controlled food challenges in CM to determine the rate of anaphylaxis to low-level exposures and the reproducibility of reaction thresholds.

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Funded in part by a UK Medical Research Council Clinician Scientist award to P.J.T. (MR/K010468/1). N.P. and P.J.T. are supported through the National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare National Health Service (NHS) Trust and Imperial College London. The views expressed in this article are those of the authors and do not necessarily reflect those of the NHS, NIHR, or the Department of Health.

Disclosure of potential conflict of interest: P. J. Turner reports grants from UK Medical Research Council, NIHR/Imperial BRC and J. M. Charitable Foundation during the conduct of the study; and personal fees from UK Food Standards Agency, Aimmune Therapeutics, Allergenis, and Aquestive Therapeutics, outside the submitted work. D. E. Campbell is a part-time employee of DBV Technologies and reports receiving grant support from National Health and Medical Research Council of Australia and personal fees from Allergenis, Westmead Fertility Centre, and Financial Markets Foundation for Children. H. A. Sampson reports receiving consultancy fees from N-Fold, LLC, DBV Technologies, and Siolta Therapeutics, grant funding from the National Institutes of Health/National Institute of Allergy and Infectious Diseases, and royalties from Elsevier. T. Katsunuma reports lecture fees from Kyorin Pharmaceutical, Maruho, and Torii Pharmaceutical outside the submitted work. J. Westerhout, W. M. Blom, and G. F. Houben report financial support from Dutch Governmental TNO Research Cooperation Funds, Netherlands, and the Food Allergy Research and Resource Program (FARRP) of the University of Nebraska. B. C. Remington reports grants from FARRP, grants and personal fees from DBV Technologies, and travel support from ILSI Europe. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication February 15, 2022; revised April 1, 2022; accepted for publication April 29, 2022.

Available online June 7, 2022.

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0091-6749

https://doi.org/10.1016/j.jaci.2022.04.035

Methods: We performed a systematic review and IPD meta-analysis of studies reporting relevant data. Authors were contacted to provide additional data and/or clarification as needed. Risk of bias was assessed using the National Institute for Clinical Excellence methodologic checklists.
Results: Thirty-four studies were included, representing data from over 1000 participants. The cumulative ED_{01} and ED_{05} (cumulative doses causing objective symptoms in 1% and 5% of the at-risk allergic population) were 0.3 (95% confidence interval [CI], 0.2-0.5) and 2.9 (95% CI, 1.6-5.4) mg, respectively. At meta-analysis, 4.8% (95% CI, 2.0-10.9) and 4.8% (95% CI, 0.7-27.1) of individuals reacting to ≤5 mg and ≤0.5 mg of CM protein had anaphylaxis (minimal heterogeneity, $I^2 = 0\%$). Then 110 individuals underwent repeat double-blind, placebo-controlled food challenges; the intraindividual variation in reaction threshold was limited to a ½-log change in 80% (95% CI, 65-89) of participants. Two individuals initially tolerated 5 mg CM protein but then reacted to this dose at a subsequent challenge, although neither had anaphylaxis.
Conclusions: About 5% of CM-allergic individuals reacting to ED_{01} or ED_{05} exposure might have anaphylaxis to that dose. This equates to 5 and 24 anaphylaxis events per 10,000 patients exposed to an ED_{01} or ED_{05} dose, respectively, in the broader CM-allergic population. Most of these anaphylactic reactions would be mild and respond to a single dose of epinephrine. (J Allergy Clin Immunol 2022;150:1135-43.)

Key words: *Allergy, anaphylaxis, cow's milk, eliciting dose, food challenge, thresholds, precautionary allergen labeling*

The reference standard for diagnosis of food allergy is the double-blind, placebo-controlled food challenge (DBPCFC),[1] although in practice most centers perform "open" unblinded food challenges (FCs) for pragmatic reasons. Increasingly, reaction threshold data from FC are being used to inform clinical management, either to help inform the starting dose for patients undergoing food allergen immunotherapy or to guide dietary liberalization in those with higher reaction thresholds (either *de novo* or after immunotherapy).[2] Separately, the use of challenge thresholds (modeled to reflect population dose distributions) is now guiding allergen risk assessment in industry—and potentially the need for "may contain" precautionary allergen labeling (PAL).[3,4]

However, while dose-distribution data for most "priority" allergens have been published,[5] there is less certainty over how reaction thresholds may change over time—that is, the reproducibility of the precise dose causing allergic symptoms in a given individual. We recently published an analysis of the reproducibility of reaction thresholds and anaphylaxis in peanut-allergic individuals,[6] and we have proposed that with the larger data sets now available pertaining to peanut, it might be possible to use peanut as a reference allergen in terms of the occurrence of anaphylaxis after low-dose exposure.[7] However, it is important to generate further evidence that this is a reasonable assumption to make, particularly for allergens more frequently associated with fatal reactions.

Cow's milk (CM) allergy is the one of the most common food allergies, but it often resolves in early childhood. In Europe, the prevalence of challenge-proven CM allergy is <1% in children up to age 2,[8] with similar estimates for North America.[9,10] However, CM allergy has a number of different phenotypes, and children with persisting CM allergy may be more at risk of severe reactions.[11] CM allergy may persist in up to 30% of CM-allergic children.[12] Over the last 25 years, CM has become the most common single cause of fatal anaphylaxis in children in the United Kingdom.[13] Epidemiologic data also show CM is a common cause of fatal and near-fatal reactions in the United States and Canada,[14-16] France,[17,18] Australia,[19] and Israel.[20] Given these data and the ubiquitous nature of dairy in the human diet, it is important to have more robust data assessing whether CM is of greater concern in terms of risk of severe reactions compared to peanut.

Therefore, in this analysis, we undertook a systematic review and meta-analysis of individual participant data (IPD)[21] to (1) update previous dose-distribution modeling for CM and provide more robust estimates for population-based eliciting doses, (2) evaluate the proportion of reactions at low levels of allergen exposure that might be classified as anaphylaxis, and (3) determine the reproducibility of individual reaction thresholds over time.

Abbreviations used

CM:	Cow's milk
DBPCFC:	Double-blind, placebo-controlled food challenge
DLS:	Dose-limiting symptoms
ED_{01}, ED_{05}, and ED_{10}:	Cumulative doses causing objective symptoms in 1%, 5%, and 10% of the at-risk allergic population
FC:	Food challenge
IPD:	Individual participant data
LOAEL:	Lowest observed adverse effect level
PAL:	Precautionary allergen labeling
WAO:	World Allergy Organization

METHODS

We undertook a systematic review of the literature to identify studies which have undertaken DBPCFC in CM-allergic individuals (adults and children). Study sponsors and/or authors were contacted and asked to provide both aggregate and (in the case of individuals who underwent repeat challenges to CM) anonymized IPD, which could then be included for meta-analysis. This review was undertaken and reported in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis)-IPD statement.[21]

Search strategy

We searched Medline and Embase for articles published between January 1984 and December 2021 that described DBPCFC to CM using the search terms "double-blind," "allergy," "challenge," and "cow's milk." There was no registered protocol for this review, but the methods and analyses were planned *a priori.* No language restrictions were made; we planned to include non–English-language papers if they met our inclusion criteria. Abstracts were independently screened by 2 authors, and disagreements were resolved by discussion. We also reviewed reference lists of included studies and review articles to identify other relevant studies.

Study selection

Inclusion criteria were as follows: (1) Participants: adults and children with suspected CM allergy; (2) Intervention: DBPCFC, conducted in a manner

consistent with PRACTALL consensus,[1] with a minimum of ≥8 subjects with objective symptoms at FC; (3) Outcomes: study-defined cumulative eliciting dose (either maximum tolerated dose or reaction threshold dose) or lowest observed adverse effect level (LOAEL); and the occurrence of anaphylaxis. Studies needed to satisfy all 3 inclusion criteria to be included. We also included historical studies incorporated into the database maintained by the Netherlands Organisation for Applied Scientific Research (TNO) and the Food Allergy Research & Resource Program at the University of Nebraska (FARRP) of reaction thresholds that were otherwise eligible for inclusion, because these had already been quality checked.[5] Where more than a single data series included the same individuals during an overlapping time period, we included the data series with the largest number of individuals where we could be certain that no duplication was present. Excluded studies are reported in Table E1 (available in the Online Repository at www.jacionline.org), together with the reason for exclusion.

Data extraction and analyses

Analyses were planned prospectively. Data relating to the no observed adverse effect level (highest dose with no objective symptoms) and LOAEL (first dose with objective symptoms) or dose causing study-defined dose-limiting symptoms (DLS) were extracted in duplicate;[22] any discrepancies identified between extracted data and published data were resolved by discussion and/or by contacting authors or study sponsors for clarification. Population dose distributions were determined as previously described, using Bayesian stacked parametric survival methods with frailty components and interval-censored failure times, also referred to as stacked model averaging.[23]

Previous published estimates for discrete ED_{05} (cumulative dose causing objective symptoms in 5% of the at-risk allergic population) for CM is 2.4 (95% CI, 1.3-5.0) mg CM protein.[5] On this basis, we then extracted for each study the number of participants experiencing objective symptoms and/or meeting study-defined challenge stopping criteria to a discrete dose of ≤5 mg CM protein, and noted whether the individual had symptoms consistent with anaphylaxis (as defined by the study authors). The different anaphylaxis definitions used are included in Table E2 in the Online Repository at www.jacionline.org. Where individual patient symptom data were available, anaphylaxis was determined according to the World Allergy Organization (WAO) 2020 consensus criteria by 2 independent investigators (P.J.T., N.P.).[24] Risk of bias was assessed using the National Institute for Clinical Excellence methodologic checklists for cohort studies.[25] Rates were pooled across studies using a generalized linear mixed model in R software (R Project; www.r-project.org) using the 'metaprop' function of the 'metafor' package, then logit transformation with a random intercept logistic regression model for the summary estimate, with a continuity correction of 0.5. This approach avoids many of the issues surrounding the use of transformations when undertaking meta-analyses of proportions.[26,27] Heterogeneity was quantified by the I^2 statistic. We conducted meta-analysis even if significant heterogeneity was seen between study estimates, as is the norm when conducting meta-analysis of proportions. The statistical program used for meta-analysis was R v4.0.3. Binomial CIs were calculated using the Clopper-Pearson interval. Statistical significance was set at 2-sided $P < .05$. Sensitivity analyses were performed to look for any difference between those studies reporting LOAELs (defined according to Westerhout et al[22]) and those using DLS on the overall pooled estimate.

In order to assess the reproducibility of challenge thresholds over time within individuals, we extracted IPD of individuals who underwent a further FC after the initial DBPCFC (conducted according to the same protocol) from relevant interventional studies—for example, participants who were randomized to a placebo control arm in studies of food allergy desensitization. The log-fold change in reaction threshold for each subject was calculated. Normality of distribution was assessed using the D'Agostino-Pearson test, and the distributions were then used in IPD meta-analysis. We included a sensitivity analysis to assess the degree of stability in reaction threshold in CM-allergic individuals reacting to lower eliciting doses (≤50 mg CM protein, approximately 1.5 mL of fresh milk). Separately, we evaluated the reproducibility in the occurrence of anaphylaxis after a repeat exposure.

Ethical approval

Ethical approval was not required because this was a *post hoc* analysis of anonymized participant data from multiple clinical trials, all of which had their own individual ethics approval.

RESULTS

Twenty-seven studies were identified as being eligible for inclusion, which, together with the 7 studies already included in the TNO-FARRP data set, resulted in a total of 34 studies (Fig 1).[5,8,28-58] Details of the individual studies appear in Table E2 and risk of bias assessment in Table E3, both available in the Online Repository at www.jacionline.org. No study had a high risk of bias or poor external validity.

Dose-distribution modeling

Data from 1002 challenges were included in the analysis. The estimated discrete and cumulative eliciting doses for the amount of CM protein predicting to cause objective symptoms in 1%, 5%, and 10% of the CM-allergic population (ED_{01}, ED_{05}, and ED_{10}, respectively) are shown in Table I and in Fig E1 in the Online Repository at www.jacionline.org. The cumulative ED_{01} and ED_{05} (95% CI) values were 0.3 (0.2-0.5) and 2.9 (1.6-5.4) mg, respectively. We therefore then assessed the rate of anaphylaxis to low-level exposures on the basis of the calculated upper 95% CIs for ED_{01} and ED_{05}—that is, 0.5 mg and 5 mg, respectively.

Anaphylaxis at low levels of allergen exposure

Overall, data from 1389 participants were available across 23 studies, of whom 105 individuals (across 14 studies) reacted (according to individual study-defined criteria) to ≤5 mg CM protein. Using estimated dose-distribution modeling (Table I), we determined the number of individuals in each study reacting to ≤5 mg and ≤0.5 mg CM protein, and the proportion who had anaphylaxis. There was no evidence of publication bias in terms of the rate of anaphylaxis in those with low-dose reactions by study size, as indicated by the funnel plot shown in Fig E2 (available in this article's Online Repository at www.jacionline.org). At meta-analysis, 4.8% (95% CI, 2.0-10.9) of those reacting to an exposure of ≤5 mg CM protein (discrete dose) had anaphylaxis (minimal heterogeneity [$I^2 = 0\%$], Fig 2, *A*). In a sensitivity analysis, we did not identify any significant differences in estimates when comparing studies that used LOAEL and those using DLS ($P = 1.0$; see Fig E3 in the Online Repository at www.jacionline.org). There were no anaphylaxis reactions reported as refractory anaphylaxis (ie, reactions that did not respond to 1 or 2 doses of adrenaline).

Seven studies included an initial challenge dose of ≤0.5 mg CM protein, of which 3 reported a total of 21 individuals who reacted to ≤0.5 mg. At meta-analysis, 4.8% (95% CI, 0.7-27.1) of reactions to ≤0.5 mg CM protein were anaphylaxis (minimal heterogeneity [$I^2 = 0\%$], Fig 2, *B*). As a result of the small number of reactions at this level of allergen exposure, no sensitivity analyses were undertaken.

Reproducibility of reaction thresholds

Five interventional studies (clinical trials of food allergen desensitization) included participants allocated to placebo treatment and who underwent repeat FC to CM at 5 to 12 months after

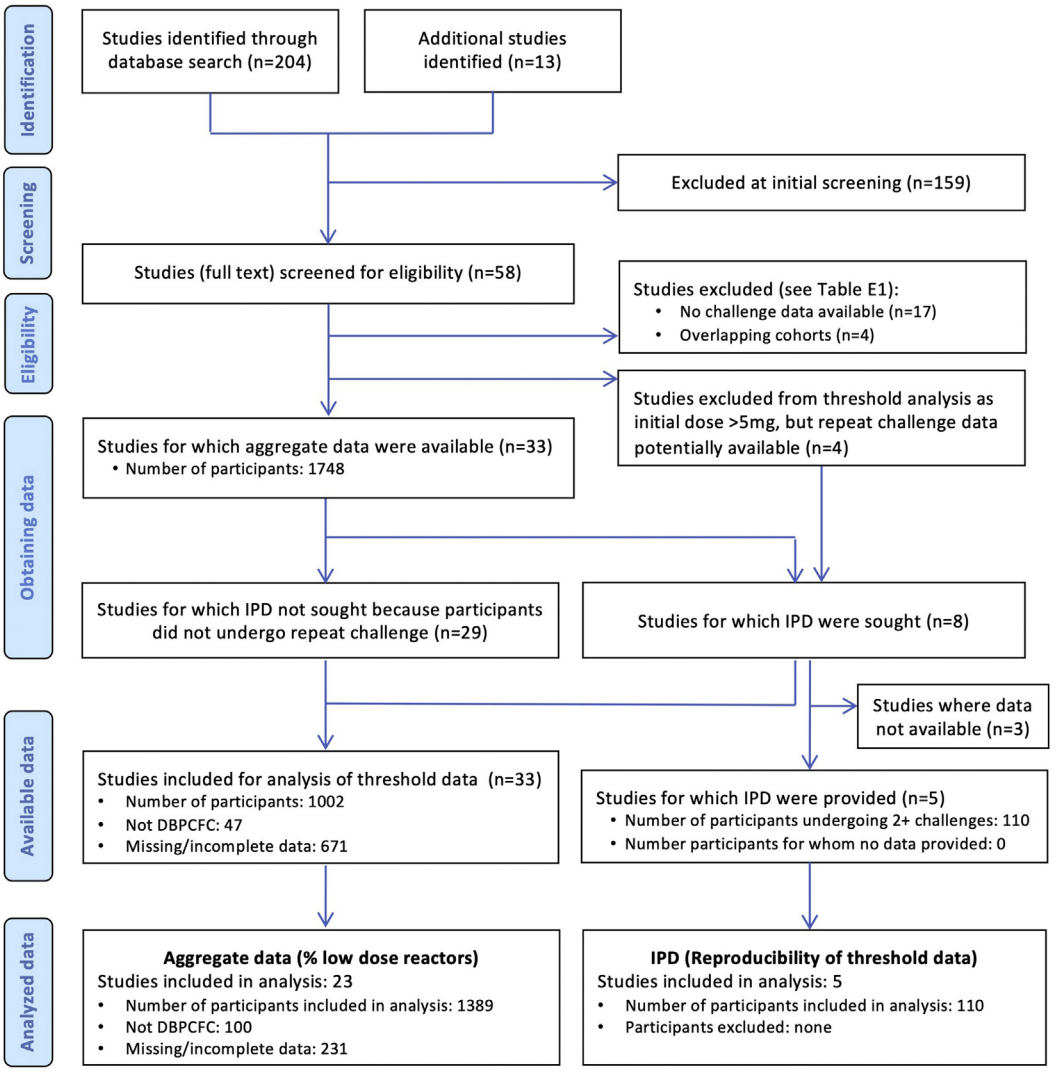

FIG 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) flow diagram.

TABLE I. Predicted eliciting dose values for CM

	mg CM protein (95% CI) for:	
Eliciting dose	**Discrete dose**	**Cumulative dose**
ED_{01}	0.2 (0.1-0.3)	0.3 (0.2-0.5)
ED_{05}	2.1 (1.2-3.8)	2.9 (1.6-5.4)
ED_{10}	6.5 (3.9-11.6)	9.3 (5.4-17.0)

initial baseline challenge.[28,31,33,41,46] Data from these participants were included in the IPD meta-analysis. The dose distributions for baseline DBPCFC in these participants are shown in Fig 3, together with the proportion reacting at each dosing level with anaphylaxis (defined according to WAO 2020 criteria[24]). The median (interquartile range) cumulative reaction threshold for this combined cohort was 144 (44-444) mg CM protein; the overall rate of anaphylaxis in this subcohort was 14.5%. Compared to published dose distributions for CM-allergic individuals,[59] there was no evidence of skewing toward a more sensitive population (Fig 3).

The distributions of log change in reaction thresholds for study participants within each included cohort are shown in Fig 4. These distributions were analyzed by IPD meta-analysis to determine the proportion of participants with a change in reaction threshold at repeat challenge, and whether this differed in patients who reacted to lower levels of CM exposure (Table II). Overall, 80% (95% CI, 65-89) of participants reacted at repeat challenge to within ±½-log increment compared to initial challenge, equivalent to within 1 dosing interval using a PRACTALL-based semi-log dosing regimen (eg, a change in threshold from 100 mg to 300 mg CM protein). Individuals who reacted to lower doses at first DBPCFC were more likely to react to higher doses at repeat challenge with a ≥½-log increase in reaction threshold. Four individuals reacted to ≤5 mg at initial challenge; all reacted to ≥5 mg at subsequent challenge. Two individuals reacted to ≤5 mg CM protein at subsequent challenge, having initially tolerated this dose; none had anaphylaxis. Reassuringly, no individuals who initially tolerated an ED_{05} level of milk exposure had anaphylaxis to an exposure of ED_{05} or less at subsequent challenge.

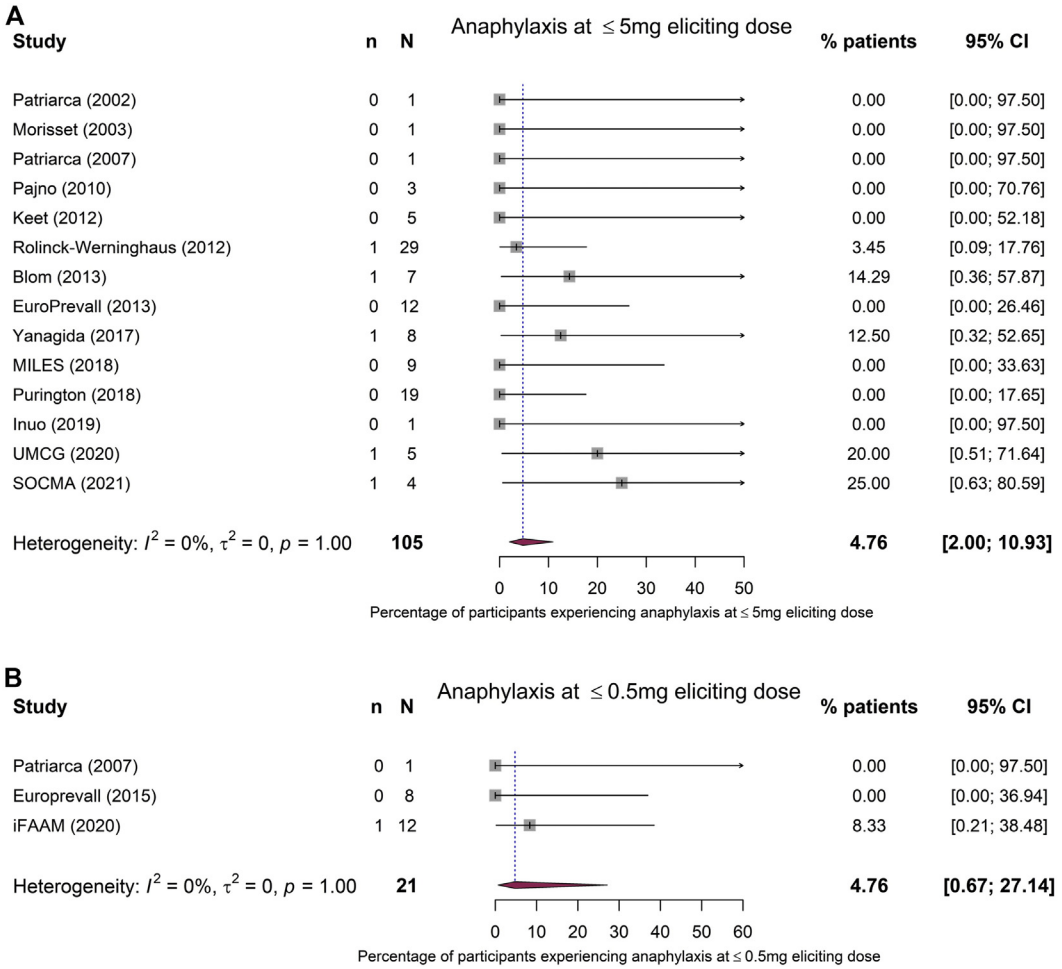

FIG 2. Meta-analysis of aggregate data from 19 studies assessing the proportion of CM-allergic individuals reacting with objective symptoms to (**A**) ≤5 mg (**B**) and ≤0.5 mg CM protein who experienced anaphylaxis at that dose.

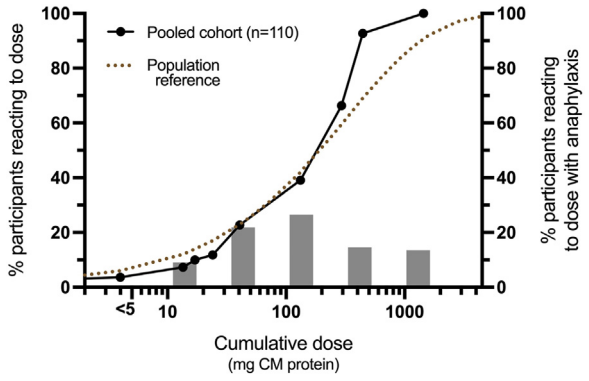

FIG 3. Dose distribution for reaction threshold at baseline DBPCFC in 110 participants included in the IPD meta-analysis (pooled cohort of 5 studies) who underwent 2 challenges. *Gray bars* indicate the proportion of subjects reacting at each dosing level with anaphylaxis (defined according to WAO 2020 criteria[24]). Population reference distribution is derived from Houben et al.[59]

Recurrence of anaphylaxis

Last, we analyzed data from participants who underwent repeat DBPCFC and who had anaphylaxis (according to WAO 2020 consensus criteria[24]) at least once. Data were available from 3 studies, yielding a total of 33 (37%) of 89 participants who had at least 1 anaphylaxis reaction. For the pooled analysis, the change in eliciting dose is shown in Fig 5. As with the above data relating to reproducibility of eliciting dose, the dose at which participants experienced anaphylaxis also varied: 4 of 33 had anaphylaxis at both FC, reacting to the same threshold on both occasions. The occurrence of anaphylaxis was not predictable, with 17 (52%) of 33 (95% CI, 34-69) participants having anaphylaxis at one FC but not at the other FC to the same or higher level of CM exposure.

DISCUSSION

In this IPD meta-analysis of threshold and symptom data from over 1000 DBPCFC to CM, about 5% of CM-allergic individuals reacting to ED_{01} and/or ED_{05} levels of CM with objective symptoms had anaphylaxis. Within the CM-allergic population, this equates to an ED_{05} exposure of CM (2.9 mg CM protein, ~0.1 mL of fresh milk) causing anaphylaxis in 24 (95% CI, 10-54.5) per 10,000 CM-allergic individuals, and an ED_{01} exposure causing anaphylaxis in 4.8 (95% CI, 0.7-27.1) per 10,000 individuals. These data are reassuringly

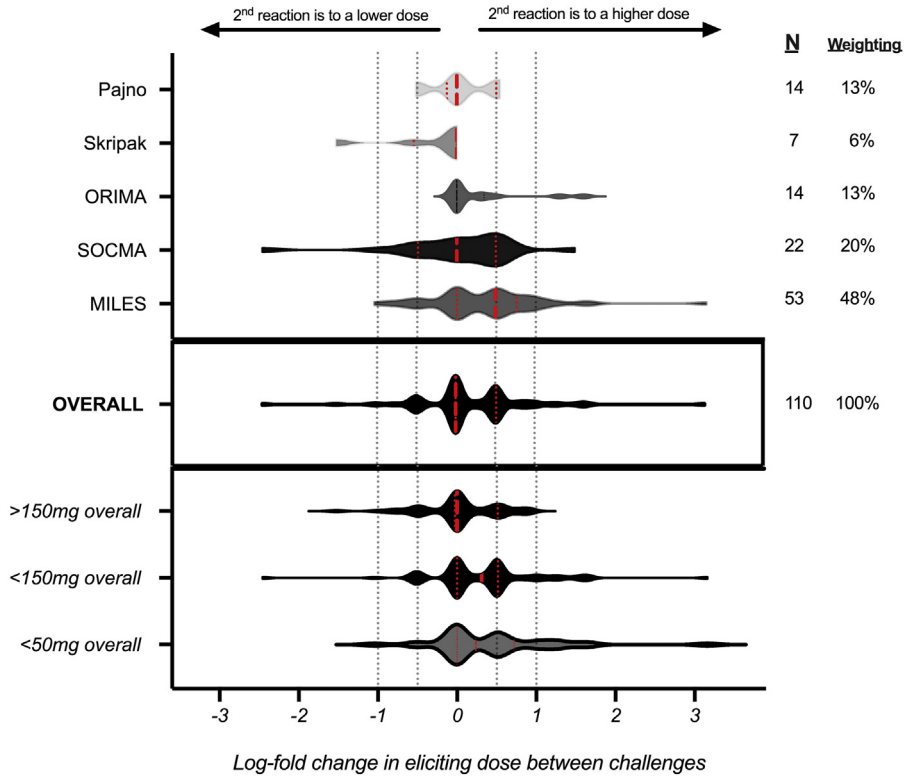

FIG 4. Violin plot of the distributions of log change in reaction thresholds (from initial to repeat DBPCFC) for study participants within each included cohort. A change of ½ log in eliciting dose is equivalent to a shift in reaction threshold by 1 dosing increment using a PRACTALL-based semi-log regimen. *Red dashed line* indicates median; *red dotted lines,* interquartile range.

TABLE II. Participants with a change or no change in threshold by IPD meta-analysis

Cumulative reaction threshold at initial challenge (mg CM protein)	Proportion of participants (95% CI) with:						
	Increase in threshold		No change in threshold	Decrease in threshold		± Max ½-log change	± Max 1-log change
	>½ log	Any		Any	>½ log		
Any (n = 110)	11% (3.8, 28)	38% (22, 57)	43% (27, 61)	17% (11, 26)	6.4% (3.1, 13)	80% (65, 89)	91% (84, 95)
>150 mg* (n = 43)	3.5% (0.05, 71)	23% (6.9, 54)	46% (27, 65)	19% (9.6, 33)	7.0% (2.3, 20)	83% (55, 95)	100% (75, 100)
<150 mg (n = 67)	17% (6.8, 38)	47% (25, 69)	38% (19, 63)	13% (7.1, 24)	4.5% (1.5, 13)	72% (57, 84)	87% (76, 93)
<50 mg (n = 36)	21% (5.4, 55)	47% (26, 69)	47% (21, 75)	8.3% (2.7, 23)	2.8% (0.4, 17)	84% (45, 97)	88% (58, 97)

Shown are proportions of participants with a change (or no change) in threshold (overall and cohorted into those with lower reaction thresholds to CM) by IPD meta-analysis. A ½-log and 1-log change in threshold are equivalent to a shift in reaction threshold by 1 or 2 dosing increments when using a challenge protocol based on PRACTALL.
*No objective symptoms to 5 mL CM.

consistent with a similar analysis for peanut using a more extensive data set of over 3000 DBPCFC,[6] providing further evidence to the contention that these data are sufficiently robust to be used for hazard characterization at a low level of allergen exposure for other priority allergens.[7]

We have previously reported that approximately 70% of peanut-allergic individuals have a degree of shift of up to ½ log in clinical reactivity (equivalent to up to 1 dosing increment when using a semi-log–based dosing regimen, such as that recommended by PRACTALL consensus—eg, from 100 mg to 300 mg protein).[6] In the current analysis, we found a similar degree of reproducibility in reaction thresholds in CM-allergic patients. These data therefore lead us to conclude that the variability in reaction thresholds to peanut previously reported may well apply to other food allergens.

Despite variations in the inclusion characteristics of the included studies and specific challenge protocols, there was only minimal heterogeneity observed at meta-analysis, providing reassurance as to the low level of uncertainty of the resulting pooled estimates. Heterogeneity was also minimal when comparing clearly defined criteria for LOAEL to DLS, with little impact on the overall pooled estimate. We have previously reported that the use of different anaphylaxis definitions across studies does not significantly affect the overall pooled estimates in sensitivity analyses for peanut challenges,[6] but were unable to confirm this by undertaking sensitivity analysis in the data set for CM because of some of the individual studies that we included did not describe which anaphylaxis definition was used.

These data provide further support for the use of eliciting doses (either ED_{05} or ED_{01}) to inform the need for PAL after a formal

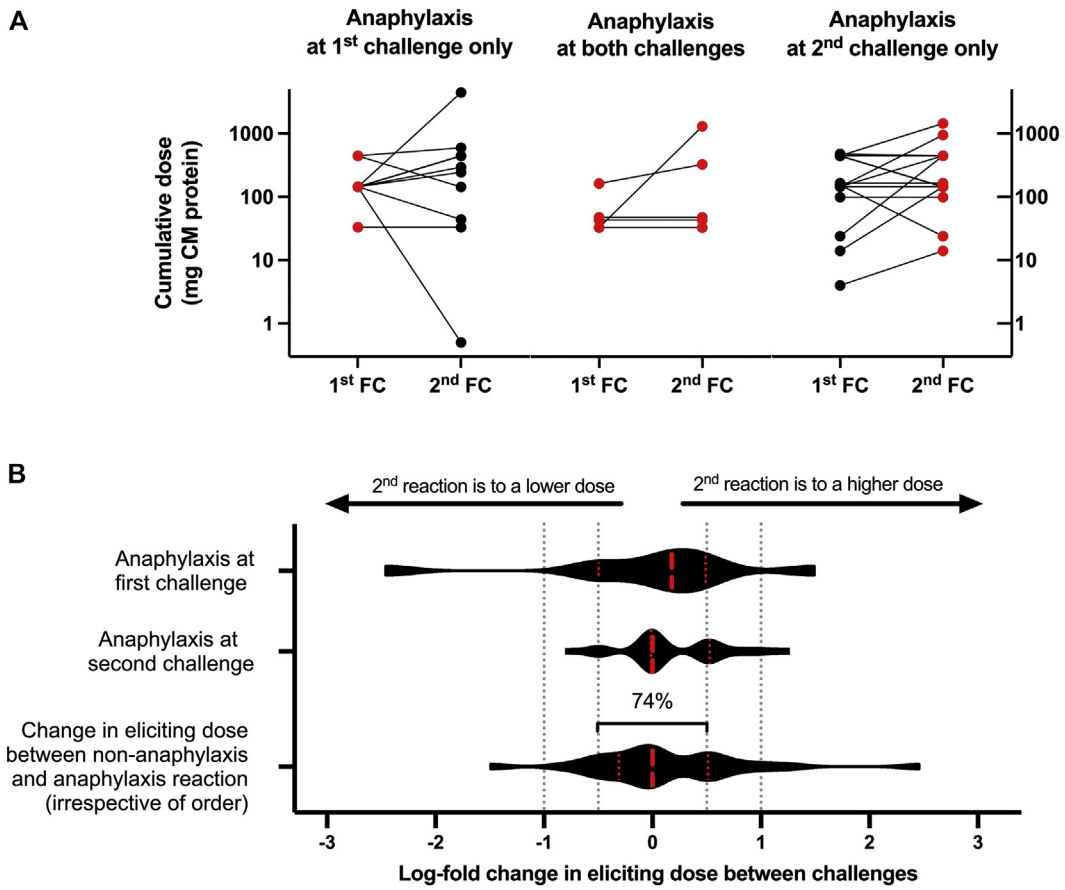

FIG 5. Change in reaction threshold in 33 participants who underwent 2 CM challenges and experienced anaphylaxis on at least 1 occasion. **A,** Absolute change in threshold. **B,** Violin plot of the distributions of log fold change in reaction thresholds between first and second challenge, unless otherwise stated. *Red dashed line* indicates median; *red dotted lines,* interquartile range.

allergen risk assessment by food businesses.[3-5] Although there will always been a tiny number of CM-allergic individuals who would still react to these levels of CM exposure, about 5% of those with anaphylaxis, these data imply that most reactions would be at the more mild end of the spectrum of anaphylaxis severity, responding to single dose of epinephrine.[60] Whether this residual risk is acceptable to patients with CM allergy needs to be determined; an ED_{01} level (rather than ED_{05}) would result in fewer individuals reacting compared to an ED_{01} level (1%, rather than 5%, of the at-risk allergic population). However, the trade-off might be more indiscriminate use of PAL, as ED_{01} levels are frequently below the limit of allergen analytical detection.[61] Food businesses might thus take a risk-averse approach and apply more PAL. This would have the paradoxical effect of increasing PAL use. In contrast, use of ED_{05} (which is achievable with existing analytics) is likely to result in less indiscriminate use of PAL, and thus significant benefits of increased food choice and consumer confidence that allergen labeling is based on a proper risk assessment procedure with adequate analytical capabilities to verify the risk management.[62,63] Given the reproducibility of challenge thresholds (particularly in low-dose reactions), it is possible to identify individuals who might react to an ED_{05} level of exposure using single-dose challenges (in much the same way as has been proposed for peanut).[29] Reassuringly, there were no CM-allergic patients who tolerated an ED_{05} level of milk exposure but then had anaphylaxis to an exposure lower than this at subsequent challenge. Identifying such "very low-dose" reactions could therefore identify these individuals to be given further advice to maintain strict CM avoidance while still allowing the majority of people to have more dietary choice.[62]

Finally, all the FCs included would have been undertaken when the participants were well and without obvious cofactors, which can affect reaction thresholds. We have previously reported that although cofactors can affect threshold, the magnitude of this impact at a population level is well within the day-to-day variation in thresholds for peanut[6] in the absence of cofactors, and now CM. However, at an individual patient level, such an impact needs to be considered if using threshold data to inform personalized allergy management advice.

Strengths and limitations

Although the analysis set included over 1000 challenges to CM conducted in 27 countries globally encompassing 5 continents, this is less than an equivalent data set for peanut previously published,[6] resulting in slightly less certainty over the estimates reported. We were unable to undertake sensitivity analyses based on participant age (as these data were not always available as a result of data confidentiality regulations), but we point out that the data include a significant proportion (at least 115, or 7.3%)

of teenagers and adults, and are therefore not limited to younger children who may be more likely to outgrow their allergy.

Of the 5 studies included in the IPD meta-analysis, at least half of the participants included were under age 10 years, and therefore it is possible that in some individuals with a higher eliciting dose at the second DBPCFC, this might have been the result of progression toward natural resolution. However, we did not observe a trend toward an increase in reaction threshold, with only 8 (7.3%) of 110 participants demonstrating a >1-log increase in reaction threshold. These data therefore provide reassurance that under typical conditions for performing DBPCFC, the clinical reaction threshold is reproducible, and thus FC remains an accurate tool to assess response to interventions for the treatment of food allergy.

Conclusions

We have reported that approximately 4% of individuals reacting to an ED_{01} or ED_{05} level of exposure to CM will have anaphylaxis to that dose; in the data set reported here, there were no cases of refractory anaphylaxis. This equates to a risk of anaphylaxis in the broader CM-allergic population of 5 and 24 anaphylaxis events per 10,000 patients exposed to an ED_{01} or ED_{05} dose, respectively. The vast majority of these anaphylaxis reactions would be at the milder end of the spectrum of anaphylaxis severity, responding to single dose of epinephrine. Although the reproducibility of reaction thresholds can vary (as for peanut), less than 5% will react to a sub-ED_{05} level after having tolerated it previously, and those who do are very unlikely to have anaphylaxis. These data will help inform future strategies to establish evidence-based approaches to allergen management, to help protect the food-allergic consumer from unintended allergen exposure.

We thank the authors of the included studies and the study sponsors for their assistance in providing data for this analysis.

Clinical implications: Approximately 5% of people with CM allergy react to ≤2.9 mg of protein (~0.1 mL of fresh milk), and 5% of those will have anaphylaxis. This equates to 24 per 10,000 milk-allergic individuals reacting to an ED_{05} exposure with anaphylaxis.

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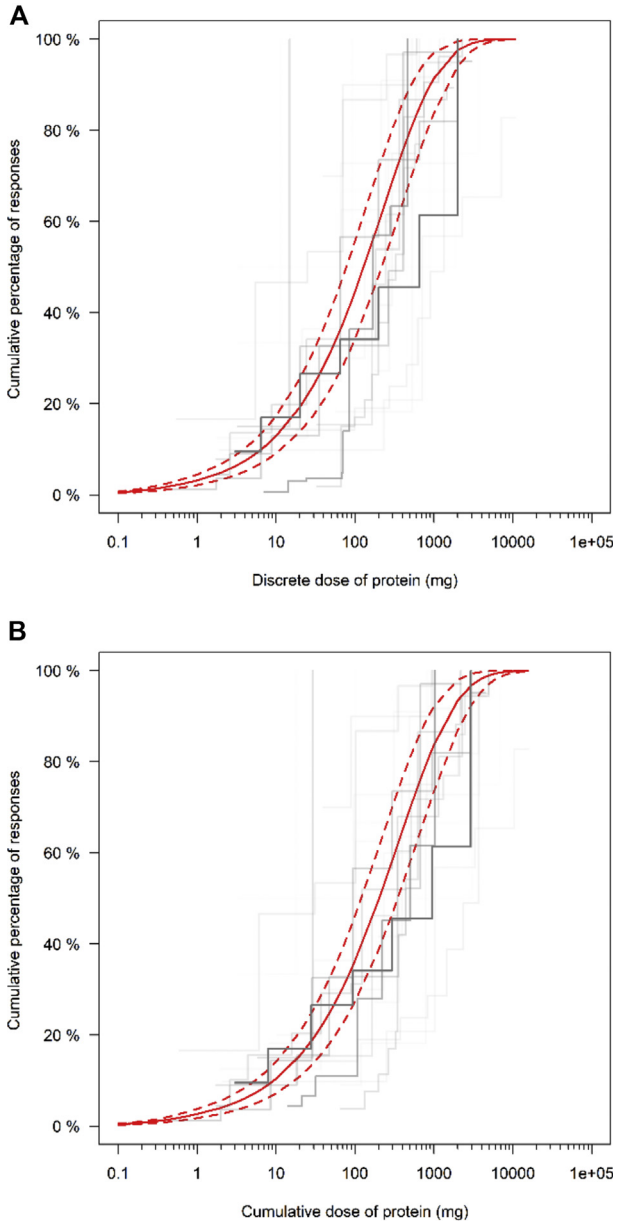

FIG E1. Eliciting dose curves from the stacked model averaged population threshold dose distributions for CM, based on discrete (**A**) and cumulative (**B**) dose data sets. Doses are expressed in milligram protein.

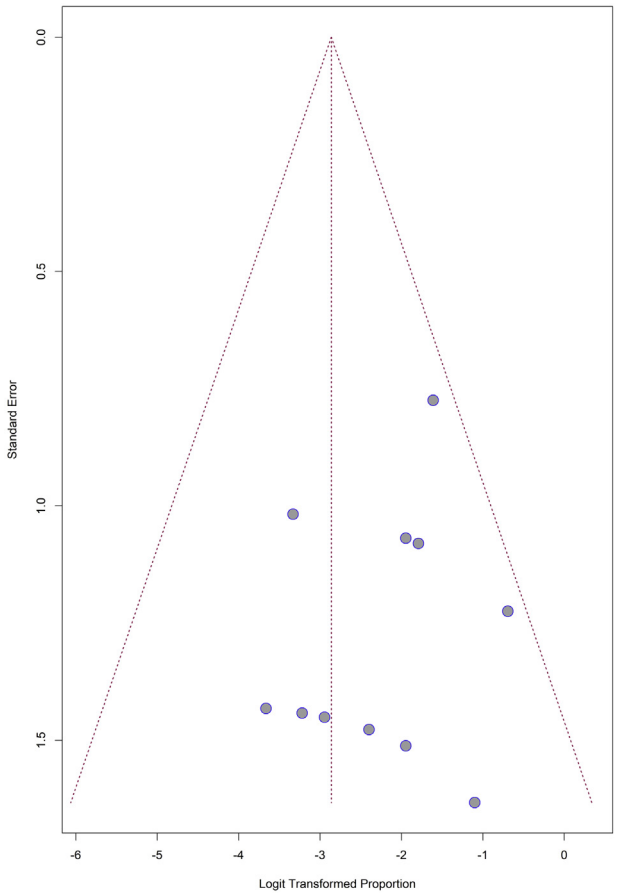

FIG E2. Funnel plot of the included studies. There was no evidence of publication bias in terms of the rate of anaphylaxis in low-dose reactors by study size.

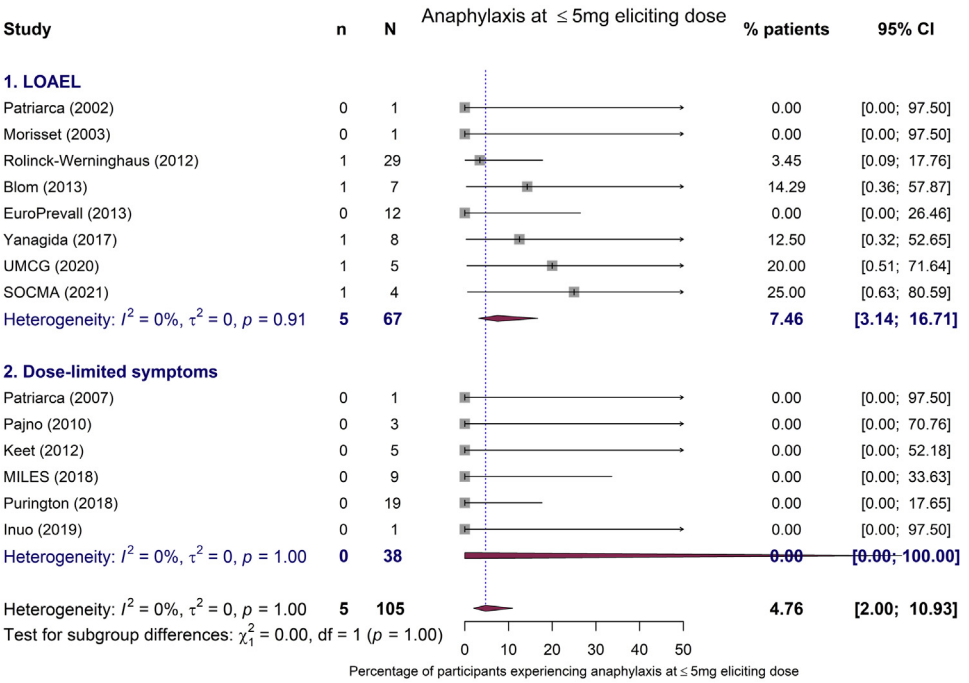

FIG E3. Meta-analysis of aggregate data from 13 studies assessing the proportion of individuals with CM allergy reacting to ≤5 mg with anaphylaxis, divided by the criteria used to define threshold (**1**) LOAEL- and (**2**) study-defined DLS.

TABLE E1. Excluded studies that otherwise met the study inclusion criteria

Study	Reason for exclusion
Ruinemans-Koerts 2019. Clin Exp Allergy 49:350-6. https://doi.org/10.1111/cea.13307	Required data not in paper and no response from authors.
De Schryver 2019. JACI Pract 7:1912-9. https://doi.org/10.1016/j.jaip.2019.02.007	Required data not in paper and no response from authors.
Capucilli 2018. Ann Allergy Asthma Immunol 121:580-7. https://doi.org/10.1016/j.anai.2018.07.018	Required data not available.
Pettersson 2018. Allergy 73:1532-40. https://doi.org/10.1111/all.13423	Unable to clarify overlap with other data sets (eg, Blom et al 2013[36]).
ElBadawy 2017. Egypt J Immunol 24:109-25.	Required data not in paper and no response from authors.
Ebrahimi 2017. Iran J Allergy Asthma Immunol 16:183-92.	Required data not available: no conversion factor to mg protein.
Andorf 2017. JACI Pract 5:1325-34. https://doi.org/10.1016/j.jaip.2017.01.016	Required data not in paper and no response from authors.
Takahashi 2016. Allergy Asthma Clin Immunol 26;12-44. https://doi.org/10.1186/s13223-016-0150-0.	Required data not in paper and unable to contact authors.
Wood 2016. JACI 137:1103-1110.e11. https://doi.org/10.1016/j.jaci.2015.10.005	Required data not in paper and no response from authors.
Okada 2015. Allergol Int 64:272-6. https://doi.org/10.1016/j.alit.2015.04.002.	Unable to clarify overlap with Yanagida 2017.[34]
Vázquez-Ortiz 2013. Clin Exp Allergy 43:92-102. https://doi.org/10.1111/cea.12012	Required data not in paper and no longer available.
Alessandri 2012. PLoS One 7:e40945. https://doi.org/10.1371/journal.pone.0040945	Required data not in paper and no longer available.
Alvaro 2012. Eur J Pediatr 171:1389-95. https://doi.org/10.1007/s00431-012-1739-z	Required data not in paper and no longer available.
Eller 2012. Ann Allergy Asthma Immunol 108:332-6. https://doi.org/10.1016/j.anai.2012.03.010	Required data not in paper and no response from authors.
Longo 2012. Eur Ann Allergy Clin Immunol 44:54-60.	Required data not in paper and no response from authors.
Martorell 2011. Clin Exp Allergy 41:1297-304. https://doi.org/10.1111/j.1365-2222.2011.03749.x	Challenge first dose >5 mg. Data regarding thresholds in participants undergoing repeat challenge not available.
Staden 2008. JACI 122:418-9. https://doi.org/10.1016/j.jaci.2008.06.002	Required data not in paper and no response from authors.
Fiocchi 2006. Clin Exp Allergy 36:311-6. https://doi.org/10.1111/j.1365-2222.2006.02428.x	Required data not in paper and no longer available.
Celik-Bilgili 2005. Clin Exp Allergy 35:268-73. https://doi.org/10.1111/j.1365-2222.2005.02150.x	Required data not in paper and no response from authors; unable to clarify overlap with Rolinck-Werninghaus 2012.[40]
Niggemann 2004. J Invest Allergol Clin Immunol 14:98-103.	Required data not in paper and no response from authors.
Fiocchi 2003. Pediatrics 112:359-62. https://doi.org/10.1542/peds.112.2.359	Overlap with Fiocchi 2003, Clin Exp Allergy.
Rancé 2002. Arch Pediatr 9(suppl 3):402s-407s. https://doi.org/10.1016/s0929-693x(02)00151-3	Required data not in paper and no longer available.
Giampietro 2001. Pediatr Allergy Immunol 12:83-6. https://doi.org/10.1034/j.1399-3038.2001.012002083.x	Required data not in paper and no response from authors.
Niggemann 2001. Pediatr Allergy Immunol 12:78-82. https://doi.org/10.1034/j.1399-3038.2001.012002078.x	Required data not in paper and no response from authors.

TABLE E2. Characteristics of included cohorts

Study (site)	No. of subjects	Age of cohort (years), median (range)	Study type	Challenge protocol (mg CM protein)	Threshold definition	Anaphylaxis definition used	Median cumulative dose (mg)	No. with symptoms to ≤5 mg discrete dose			No. included in analysis for:		ED data censored	
								Objective symptoms	Study-defined anaphylaxis					
									N	Symptoms	ED	Anaphylaxis	Right	Left
SOCMA 2021[28] (UK, Spain)	83	10 (6-18)	Immunotherapy	DBPCFC, 20-30 min intervals (½, 3, 10, 30, 100, 300, 1000, 3000)	DLS	WAO	143.5	4	1	Throat tightness, cough	83	83*	4	1
iFAAM 2020[29] (UK, Spain, Ireland)	172	1.0 (0.2-16)	Diagnostic	Single-dose FC (0.5 mg) (DBPCFC in 50%)	LOAEL	WAO	433	12	1	Bilateral wheeze, erythema	†	172		
UMCG 2020[5] (Netherlands)	53	2.3 (0.3-17.6)	Diagnostic	DBPCFC before 2007 (1.75, 3.5, 14, 70, 130, 350, 570); DBPCFC after 2007 (0.6, 3, 10, 30, 100, 300, 1000)	LOAEL	CVS/LRS	437.5	5	1	Vocal hoarseness with mild dyspnea	53	53	12	5
UMCU 2020[5] (Netherlands)	15	15 adults (age ≥18 y)	Diagnostic	DBPCFC, 30 min intervals (0.3, 3, 30, 90, 300, 900, 3000, 9000)	LOAEL	CVS/LRS	1560	1	DNA		15		4	1
Inuo 2019[30] (Japan)	25	4.3 (1-9)	Diagnostic	DBPCFC, 30 min intervals (first dose 5 mg)	DLS	CVS/LRS	61	1	0		§	25		
Maeda 2021[31]	28	Mean 5.4 (range 3-12)	Immunotherapy	DBPCFC, 15 min intervals (33, 66, 66, 165, 330, 660, 990, 990)	DLS	Sampson IV	66	0	0		¶	28		
Purington 2018[32] (USA)	67	Children + adults	Diagnostic	DBPCFC, 15 min intervals (1.7, 5, 20, 50, 100, 100, 100, 125)	DLS	NIAID	327	19	0		§	51		
MILES 2018[33] (USA)	198	Children 2-11	Immunotherapy	DBPCFC, 20 min intervals (1, 3, 10, 30, 100, 300)	DLS	Consistent with WAO‡	144	9	0		¶	198		
Yanagida 2017[34] (Japan)	164	Median 8.6 (children ≥5 y)	Diagnostic	DBPCFC, 30 min intervals (71, 142, 283, 425, 779)	LOAEL	Sampson IV	840	8	1	GI symptoms, cough, dyspnea	164	164	0	15
Gushken 2013[35] (Brazil)	58	5.3 (1.1-15)	Diagnostic	DBPCFC, 15 min intervals (33, 132, 330, 462, 660, 792, 792)	LOAEL	CVS/LRS	450	NA	NA		39	39	0	18
Blom 2013[36] (Netherlands)	93	2.2 (0.6-13.9)	Diagnostic	DBPCFC, 30 min intervals (1.75, 3.5, 14, 70, 350, 1750, 2190)	LOAEL	CVS/LRS	389	7	1	Vocal hoarseness, mild cough, pruritus, face angioedema	78	78	8	4
Dambacher 2013[37] (Netherlands)	21	0.7 (0.3-11)	Diagnostic	DBPCFC, 20 min intervals (18, 180, 360, 540, 720, 1080, 1620)	LOAEL	CVS/LRS	1620	0	0		21	21	3	3
Lee 2013[38] (South Korea)	31	Mean 0.7	Immunotherapy	DBPCFC, 30 min intervals (3.3, 16.7, 33, 67, 167, 333)	DLS	CVS/LRS	165	0	0		§	31		

(Continued)

TABLE E2. *(Continued)*

Study (site)	No. of subjects	Age of cohort (years), median (range)	Study type	Challenge protocol (mg CM protein)	Threshold definition	Anaphylaxis definition used	Median cumulative dose (mg)	No. with symptoms to ≤5 mg discrete dose			No. included in analysis for:		ED data censored	
								Objective symptoms	Study-defined anaphylaxis					
									N	Symptoms	ED	Anaphylaxis	Right	Left
EuroPrevall 2013[8] (Europe, 16 countries)	69	2 adults, 67 children median 1.2 y	Diagnostic	DBPCFC, 20 min (0.003, 0.03, 0.3, 3, 30, 90, 300, 900, 3000)	LOAEL	CVS/LRS	123	12	0		§	69		
Keet 2012[39] (USA)	30	8 (6-15)	Immunotherapy	DBPCFC, 15 min intervals (0.1, 1 10, 40, 100, 400, 800, 1200)	DLS	Not stated	51	5	0		30	30	0	0
Rolinck-Werninghaus 2012[40] (Germany)	305	1 (0.2-16)	Diagnostic	DBPCFC [73%], open FC [27%] (3, 10, 30, 100, 300, 1000, 3000)	LOAEL	Sampson IV	1443	29	1	Anaphylaxis (respiratory symptoms)	305	305	0	29
Pajno 2010[41] (Italy)	30	10 (4-13)	Immunotherapy	DBPCFC 30 min intervals (3, 9, 30, 90, 300, 900, 3000)	DLS	CVS/LRS	100	3	0		§	30		
Caminiti 2009[42] (Italy)	11	Mean ~8 (range 5-10)	Immunotherapy	DBPCFC, 20 min intervals (3, 9, 30, 90, 300, 900, 3000)	DLS	CVS/LRS	145	0	0		11	11	0	0
Orhan 2009[43] (Turkey)	4	8.5 (7-9)	Diagnostic	DBPCFC, 15 min intervals (165, 330, 1320, 2475, 4950)	LOAEL	Not stated	3050	NA	NA		4		0	0
Longo 2008[44] (Italy)	60	Mean 8 (range 5-17)	Immunotherapy	DBPCFC, 20 min intervals (0.75, 1.5, 3, 6, 12, 24)	LOAEL	Not stated	12	9	DNA		60		0	9
Lam 2008[45] (Netherlands)	10	40 (17-68)	Diagnostic	DBPCFC, 30 min intervals (0.3, 3, 30, 90, 300, 900, 3000, 9000)	LOAEL	CVS/LRS	900	0	0		‖	10		
Skripak 2008[46] (USA)	20	9.5 (6-15)	Immunotherapy	DBPCFC, interval not stated (40, 100, 400, 800, 1200)	LOAEL	Not stated	40	NA	NA		20		0	14
Staden 2007[47] (Germany)	24	2.9, 3.6, 5.8	Immunotherapy	DBPCFC, 30 min intervals (3, 10, 33, 100, 330, 1000, 3300)	LOAEL	Not stated	99	0	0		3		0	0
Morisset 2007[48] (France)	60	Mean 2.2 (range 1.1-6.5)	Immunotherapy	Single blinded, 20 min intervals (3.3, 6.6, 16.5, 50, 165, 495, 1320)	LOAEL	CVS/LRS	1650	0	0		11	11	0	1
Patriarca 2007[49] (Italy)	11	5.5 (3-15)	Immunotherapy	DBPCFC, 30 min intervals (0.033, 0.165, 0.33, 0.66, 1.65, 3.3, 6.6, 16.5, 33, 132, 264, 528, 1056, 1980)	DLS	CVS/LRS	102	1	0		§	11		
Devenney 2006[50] (Sweden)	4	3.3 (1.8-5.5)	Diagnostic	DBPCFC, 20 min intervals (3.3, 16.5, 165, 500, 1000)	LOAEL	Not stated	DNA	At least 1	DNA		2		0	1

(Continued)

TABLE E2. *(Continued)*

Study (site)	No. of subjects	Age of cohort (years), median (range)	Study type	Challenge protocol (mg CM protein)	Threshold definition	Anaphylaxis definition used	Median cumulative dose (mg)	No. with symptoms to ≤5 mg discrete dose			No. included in analysis for:		ED data censored	
								Objective symptoms	Study-defined anaphylaxis		ED	Anaphylaxis	Right	Left
									N	Symptoms				
Flinterman 2006[51] (Netherlands)	11	4.0 (1.8-10.3)	Diagnostic	DBPCFC, 20-30 min intervals (180, 360, 540, 720, 1080, 1620)	LOAEL	Not stated	540	NA	NA		11		0	3
Fiocchi 2003[52] (Italy)	18	5 (0.5-9.8)	Diagnostic	DBPCFC, 30 min intervals (198, 396, 792, 1584)	LOAEL	Not stated	504	NA	NA		12		0	5
Morisset 2003[53] (France)	59	52 children, 7 adults	Diagnostic	Blinded FC, 20 min intervals (3.3, 6.6, 16.5, 50, 165, 495, 1320)	LOAEL	CVS/LRS	DNA	1	0		3	59	0	1
Patriarca 2002[54] (Italy)	8	10.5 (5-15)	Immunotherapy	DBPCFC, 30 min intervals (0.33, 3.3, 16.5, 33, 165, 333, 1000)	LOAEL	CVS/LRS	218	1	0		8	8	0	0
Baehler 1996[55] (Canada)	69	Mean 3	Diagnostic	DBPCFC, 15 min intervals (1.8, 16.5, 33, 83, 165, 330, 660, 1000)	LOAEL	CVS/LRS	180	0	0		10	10	0	0
Norgaard 1992[56] (Denmark)	4	Adults 29-44 y	Diagnostic	DBPCPFC, 30 min intervals (first dose 16.5 mg)	LOAEL	Not stated	1700	0	0		3	3	0	1
Høst 1988[57] (Denmark)	5	1.2 (1.0-3.3)	Diagnostic	DBPCPFC, 120 min intervals (first dose 165 mg)	LOAEL	Not stated	165	NA	NA		3		0	2
Hill 1984[58] (Australia)	100	Mean 1.4, 95% ≤3 y	Diagnostic	Open FC, 30 min intervals (330, 660, 990, 2000, 4000, 8000)	LOAEL	Not stated	594	NA	NA		53		0	28

All doses are expressed as mg CM protein. *CVS,* Cardiovascular; *DNA,* data not available; *GI,* gastrointestinal; *LRS,* lower respiratory symptoms; *NA,* not applicable; *NIAID,* National Institute of Allergy and Infectious Diseases.
*Overlap with iFAAM 2020, so data only used to inform dose distribution for eliciting dose, severity analysis for ≤5 mg exposure, and reproducibility analyses.
†Overlap with SOCMA 2021, so data used to inform severity analysis for ≤1 mg CM exposure only.
‡Individual participant symptom data were available in these studies and were used to reassign the occurrence of anaphylaxis (or not) using WAO 2020 criteria.
§Detailed symptom/threshold data not available, so data used to inform severity analysis only.
¶Data used to inform severity and reproducibility analyses only.
‖Data included in UMCU 2020 adult data and thus only used for severity analysis.

TABLE E3. Risk of bias in included studies

Study	Design	Interval between FC	Selection bias*	Attrition bias	Detection bias	Internal validity	External validity†	Comments
SOCMA 2021[28]	Interventional RCT	6 mo	Low	Low	Low	++	+	Inclusion of cED ≤1443 mg so potential skewing of population.
iFAAM 2020[29]	Diagnostic	NA	NA	NA	Low	++	++	Minimum 1 h interval to assess tolerance to 0.5 mg CM.
UMCG 2020[5]	Diagnostic	NA	NA	NA	Low	+	++	
UMCU 2020[5]	Diagnostic	NA	NA	NA	Low	+	+	
Inuo 2019[30]	Dose-ranging study	NA	NA	Low	Low	+	+	
Maeda 2021[31]	Interventional RCT	12 mo	Low	Low	Moderate	+	+	15 min intervals between challenge doses.
Purington 2018[32]	Diagnostic	NA	NA	NA	Moderate	+	-	Participants tolerating ≥500 mg excluded from analysis; 15 min intervals between challenge doses.
MILES 2018[33]	Interventional RCT	12 mo	Low	Low	Low	++	+	Inclusion of cED ≤444 mg, so potential skewing of population.
Yanagida 2017[34]	Diagnostic	NA	NA	NA	Moderate	+	++	
Gushken 2013[35]	Diagnostic	NA	NA		Moderate	+	±	15 min intervals between challenge doses.
Blom 2013[36]	Diagnostic	NA	NA	NA	Low	++	++	No information on self-selection due to subjects declining to participate.
Dambacher 2013[37]	Diagnostic	NA	NA	NA	Low	+	±	
Lee 2013[38]	Interventional	NA	NA	NA	Low	+	±	
EuroPrevall 2013[8]	Diagnostic	NA	NA	NA	Low	++	+	80 challenges eligible; data available for 67 subjects.
Keet 2012[39]	Interventional RCT	NA	NA	NA	Moderate	++	±	15 min intervals between challenge doses.
Rolinck-Werninghaus 2012[40]	Diagnostic	NA	NA	NA	Low	++	++	
Pajno 2010[41]	Interventional RCT	18 wk	Low	Low	Low	+	±	
Caminiti 2009[42]	Interventional	NA	NA	NA	Low	+	±	
Orhan 2009[43]	Diagnostic	NA	NA	NA	Moderate	+	±	15 min intervals between challenge doses.
Longo 2008[44]	Interventional RCT	NA	NA	Low	Moderate	++	+	
Lam 2008[45]	Diagnostic	NA	NA	NA	Low	+	±	
Skripak 2008[46]	Interventional RCT	23 wk	Low	Low	Unclear	±	±	Dosing interval not stated.
Staden 2007[47]	Interventional	NA	NA	NA	Low	+	±	
Morisset 2007[48]	Interventional	NA	NA	NA	Moderate	+	±	
Patriarca 2007[49]	Interventional	NA	NA	NA	Low	+	±	
Devenney 2006[50]	Diagnostic	NA	NA	NA	Moderate	+	±	
Flinterman 2006[51]	Diagnostic	NA	NA	NA	Low	+	±	
Fiocchi 2003[52]	Dose-ranging study	NA	NA	NA	Low	+	±	
Morisset 2003[53]	Diagnostic	NA	NA	NA	Moderate	+	+	
Patriarca 2002[54]	Interventional	NA	NA	NA	Low	+	±	
Baehler 1996[55]	Diagnostic	NA	NA	NA	Moderate	+	+	15 min intervals between challenge doses.
Norgaard 1992[56]	Diagnostic	NA	NA	NA	Low	+	±	
Høst 1988[57]	Diagnostic	NA	NA	NA	Low	+	±	
Hill 1984[58]	Diagnostic	NA	NA	NA	Unclear	+	±	

cED, Cumulative eliciting dose; *NA*, not applicable; *RCT*, randomized controlled trial. Validity is expressed as follows: ++ indicates all or most of the criteria have been fulfilled, and where not, the conclusions are very unlikely to be altered; +, some criteria have been fulfilled, and where not fulfilled or adequately described, the conclusions are unlikely to be altered; and ±, few or no checklist criteria fulfilled.

*Selection bias refers to possible differences in subject allocation between intervention and control groups. This was not relevant for studies that were not used for the IPD meta-analysis to assess reproducibility of reaction thresholds at DBPCFC.

†External validity assesses whether selection bias affects whether study data are generalizable to the overall CM-allergic population.