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Safety and predictors of adverse events during oral immunotherapy for milk allergy: severity of reaction at oral challenge, specific IgE and prick test

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Summary

Background Strict avoidance is the only accepted management for cow's milk (CM) allergy. CM oral immunotherapy (CM-OIT) is under investigation.

Objectives To evaluate long-term safety of CM-OIT. To identify clinical/immunological predictors of adverse events.

Methods Prospective longitudinal epidemiological intervention study. CM-allergic children aged 5–18 underwent a Spanish-approved CM-OIT protocol without premedication. Clinical data, skin prick test (SPT) and specific IgE (sIgE) at baseline and 1 year after OIT were registered. All dose-related reactions, treatments needed and cofactors involved were recorded. Through survival analysis, we studied the cumulative probability of reactions resolution over time and clinical/immunological risk factors of reactions persistence.

Results 81 children were recruited. Mean follow-up was 25 months. 95% of children suffered reactions, 91% of which affected a single organ. Reactions were heterogeneously distributed: (a) 60 children (75%) had occasional symptoms which ceased over time. 86% of them reached complete desensitization (200 mL). (b) 20 children (25%) suffered frequent (78% of total reactions), more severe and unpredictable reactions, which persisted during follow-up or led to withdrawal (6 cases). Reactions persistence was associated with a higher frequency and severity. Kaplan–Meier estimate revealed a cumulative probability of reactions resolution of 25% at 3 months (95% CI: 1.9–4.1) and 50% (95% CI: 6.1–9.9) at 8 months based on all patients. Cox proportional hazards multivariate regression model identified 3 variables ($\text{CM-sIgE} \geq 50 \text{ KU L}^{-1}$, $\text{CM-SPT} \geq 9 \text{ mm}$ and Sampson's severity grades 2, 3 and 4 at baseline food challenge) as independent risk factors of reactions persistence. The combination of 2 or 3 of these factors involved hazard ratios to develop persistent reactions of 2.26 (95% CI: 1.14–4.46; $P = 0.019$) and 6.06 (95% CI: 2.7–13.7; $P < 0.001$), respectively.

Clinical implications CM-OIT was insufficiently safe in 25% of children. The above-mentioned clinical and immunological parameters would help clinicians to identify highly reactive patients before CM-OIT. In them, individualized schedules and premedication should be considered.

Keywords anaphylaxis, cow's milk allergy, cow's milk sIgE, cow's milk skin prick test, food allergy, omalizumab, oral desensitization, oral immunotherapy, safety, specific oral tolerance induction, survival analysis

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Introduction

Food allergy is increasing in developed societies, being cow's milk (CM) allergy the most frequent in children. Resolution of CM allergy throughout infancy and

childhood is common [1–5], but recent studies suggest an increase in persistence [6, 7]. The only accepted management in food allergy is the complete avoidance of the culprit food [1, 8, 9] to prevent reactions, which can be life-threatening. Milk elimination involves a

wide dietary restriction, which leads to negative nutritional, social, psychological and economical consequences [1, 10, 11]. Moreover, because of milk's ubiquity, avoidance cannot be always guaranteed and accidental reactions occur [8].

Due to the high prevalence of food allergy and its implications, food allergy management has emerged as a developing research field [9]. With this respect, growing interest exists concerning oral immunotherapy (OIT)/desensitization procedures. The main goal of OIT is the rise in the threshold dose which triggers symptoms, protecting patients against reactions on accidental exposure. Secondly, it will allow the inclusion of the culprit food on the patients' diet, with the subsequent benefit in nutrition and quality of life [9]. Previous studies on CM-OIT have shown efficacy rates of 66%–93% [12–27]. Nevertheless, still today there is a lack of sufficient evidence regarding long-term efficacy, safety, impact on quality of life and cost-effectiveness. Thus, CM-OIT is considered nowadays an experimental treatment [8, 10, 28, 29].

The Spanish Society of Paediatric Allergy and Clinical Immunology (SEICAP) developed an OIT protocol for CM allergy in 2006 [20]. Considering the above-mentioned promising results on efficacy and encouraged by the expected benefit in our CM-allergic children, our Department implemented the SEICAP CM-OIT protocol in October 2006 as a prospective longitudinal study. Our efficacy results on 66 children under CM-OIT were recently published, reaching complete desensitization in 77.2% of cases [30]. The present study aims at evaluating the global safety of CM-OIT procedure and identifying clinical and immunological predictors of adverse events. We provide thorough clinical information about long-term safety and role of cofactors in 81 children on CM-OIT.

Objectives

The aim of this study is to evaluate safety of CM-OIT and to evaluate clinical and immunological parameters potentially associated with the development of adverse events during CM-OIT.

Methods

Study design

Prospective longitudinal epidemiological intervention study performed from October 2006 to May 2011 in the Paediatric Allergy and Clinical Immunology Department of Sant Joan de Déu tertiary referral Hospital, Barcelona, Spain. Patients were recruited sequentially. This study was approved by the Hospital's Ethics Committee (approval number: PIC-77-10).

Patients

Inclusion criteria.

- CM-allergic children aged 5–18 years.
- Confirmation of persistent CM allergy within the month before CM-OIT start (T0) by means of (1) positive clinical history, plus (2) positive double-blind placebo-controlled food challenge (DBPCFC) to CM and (3) specific IgE (sIgE, CAP-Phadia, Uppsala, Sweden) and skin prick test (SPT, Stallergenes Ibérica, S.A.) to CM and to major cow's milk proteins (α -lactalbumin, β -lactoglobulin and casein) above 0.35 KU L^{-1} and 3 mm, respectively.
- We ensured adequate familial comprehension and implication. Signature of informed consent was obtained.

Exclusion criteria. Exclusion criteria for enrolment in CM-OIT were severe or uncontrolled asthma according to GEMA guideline [31], severe atopic dermatitis according to SCORAD [32] and malignant, mental or cardiovascular diseases.

Only patients included in the CM-OIT protocol for at least 6 months were eligible for safety analysis. However, children who withdrew CM-OIT due to repeated reactions were included in the descriptive safety analysis regardless of the follow-up period.

Procedures

Cow's milk DBPCFC. Increasing doses of CM with hourly interval were given during hospitalization (1.2–2.5–5–10–25–60–145 mL) until IgE-mediated symptoms appeared. Reactions severity was assessed through the 'Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms' by Sampson, which could be summarized as follows: grade 1 for localized skin symptoms; grade 2 for mild rhinoconjunctivitis, nausea or isolated vomiting; grade 3 for intense rhinoconjunctivitis, repetitive vomiting or anxiety; grade 4 for asthma symptoms, hoarseness, diarrhoea, mild hypotension or dysrhythmia; grade 5 for loss of bowel control, respiratory/cardiac arrest, loss of consciousness, severe bradycardia or hypotension [33]. We included abdominal pain as grade 2 reaction if mild and grade 3 if moderate/severe.

When symptoms occurred before completing a dose, the exact eliciting dose was noted. Pasteurized whole milk containing 3 g of milk protein per 100 mL was used for DBPCFC and CM-OIT.

Cow's milk oral immunotherapy protocol. The CM-OIT protocol proposed by SEICAP was followed [20, 30]. The starting induction phase (IP) consisted of a period

of increasing dosage over 16 weeks: patients were initially admitted to hospital and received hourly 1–2–4–8 and 16 mL of CM diluted 1/100 with water (first day) followed by 1.5–3–6 and 12 mL of CM diluted 1/10 plus 2.5 mL of undiluted CM (second day). Patients were then discharged continuing with the last tolerated dose, once daily, throughout the week. Patients, whose threshold dose at DBPCFC was greater than 25 mL, remained a third $\pm$ fourth day at hospital receiving further increasing CM doses. Subsequently, weekly increases were given at the outpatient clinic, following this schedule: 4–6–8–10–12–15–20–25–30–40–50–75–100–125–150–200 mL. Finally, desensitization to the maximal CM-tolerated dose without reaction was confirmed through an open food challenge. Thereafter, this dose was given once daily uninterruptedly (maintenance phase, MP). Asthmatic patients under anti-asthmatic therapy followed their treatment during the study. No other premedication was given. Exceptionally, a girl was included despite severe asthma under satisfactory treatment with omalizumab, because of a past medical history of very frequent grade 4 accidental reactions to CM traces or inhaled milk. Families were instructed in the recognition and treatment of reactions according to EAACI guideline [34]. Oral antihistamines were indicated for isolated rhinitis/conjunctivitis or cutaneous symptoms; inhaled salbutamol for isolated mild asthma and intramuscular epinephrine for moderate/severe anaphylaxis. Potential cofactors were explained to avoid them [exercise, fasting, non-steroidal anti-inflammatory drugs (NSAIDs)] or minimize their impact. In case of intercurrent infectious illness, CM dose was reduced from 10 to 50%, depending on the severity of symptoms, until recovery. If dose-related reactions appeared, CM dose was reduced 1 step if mild (grade 2) and 2 steps if moderate (grade 3/4) for 1 week and the scheduled increase was then attempted. Patients with frequent reactions received smaller and slower increases (50% of scheduled increases every 2 weeks). Families could contact the medical team by telephone in case of incidences or doubts.

Variables and data sources

Descriptive variables. Clinical and immunological data were collected from medical charts: age, gender, pre-existing asthma and severity [31], atopic dermatitis, other food allergies, other comorbidities, threshold CM dose and severity of reaction at baseline DBPCFC, severity of the most severe previous reaction reported, CM and casein-sIgE and SPT levels before CM-OIT (T0) and 1 year after (T1), maximum CM-tolerated dose after IP and time required to achieve it. Complete desensitization was established at 200 mL of CM, as in other

studies [14, 16, 18–20], whereas partial desensitization at 20–200 mL.

For safety data record, children were evaluated weekly by paediatric allergists during IP and, at least, every 3 months during MP. Each patient had a study notebook where dose-related reactions and treatments needed, either at hospital or at home, during IP or MP, were registered. Each reaction was assessed for severity according to the above-mentioned Sampson's Grading [33]. Reactions were also classified as multisystemic or affecting a single organ. Coincidence with cofactors was investigated for each reaction, including unexpected elicitors repeatedly observed. Other comorbidities ascribed to CM, the need of maintained dose reduction and withdrawals (and their cause) were also recorded.

Statistics

Univariate comparative analysis through parametrical and non-parametrical test was performed to assess differences in safety data and clinical/immunological parameters between withdrawals and groups with persistent or transient reactions. The Spearman correlation coefficient was used to measure the strength of the linear relationship between quantitative predictors (sIgE, SPT, threshold at DBPCFC) and reactions (graded by severity) in IP and MP. We analysed survival using Kaplan–Meier estimator and Cox proportional hazards regression model. The event of interest was defined as reactions resolution (absence of further reactions for a minimum period of 4 months since the last reaction registered). We used univariate analysis, and subsequently, multivariate Cox regression model to assess differences in reactions resolution by clinical and immunological variables. SPSS 19.0 (SPSS Inc., Chicago, IL, USA) was used. A two-tailed $P < 0.05$ was considered significant.

Results

Patients

Eighty-one children underwent CM-OIT from 2006 to 2011. None was lost to follow-up. Fifty-eight children (71.6%) reached complete desensitization (≥ 200 mL) and 17 (20.9%), partial desensitization (20–200 mL). Ninety-five per cent of children had at least one dose-related reaction during CM-OIT. To evaluate safety, we first centred our analysis in the time needed for each patient to overcome CM-dose-related reactions on a survival analysis basis. Accordingly, patients were classified into two groups: 60 patients (75%, named group G1) overcame dose-related reactions during follow-up, whereas 14 children suffered persistent reactions and 6 children withdrew CM-OIT due to adverse events (group

G2) (Fig. 1). The division in G1 and G2, according to reactions resolution or persistence over time, was used for downstream analysis.

Median follow-up was 25 months (range: 6.2–52 months), 4.5 months (range: 1–14 months) for IP and 20 (0–47 months) for MP. A total of 61 635 CM doses were administered, 12 510 in IP and 49 125 in MP. Follow-up time was similar in patients with transient reactions (26.5 months; range: 6–52) and those with persistent reactions (23.5 months; range: 8–47; $P = 0.194$).

Descriptive data of the entire sample are represented in Table 1. Thirty-six patients (44.4%) had baseline CM and/or casein-sIgE ≥ 50 KU L⁻¹.

Dose-related reactions: number, distribution, severity, symptoms and cofactors involved

Four thousand and ninety-five reactions were registered (1/15 doses), 1534 in IP (1/8 doses) and 2561 in MP (1/19 doses). Regarding the percentage of doses with reaction per child in IP and MP, the most remarkable observations were as follows: (a) reactions decreased from IP to MP in terms of frequency and children involved (IP vs. MP, reactions/dose/child: 75th percentile: 13% vs. 4.3%; 50th percentile: 6.6% vs. 0.6%; 25th percentile: 2.6% vs. 0%; $P < 0.001$). (b) There was a wide heterogeneity among children in number of reactions suf-

fered, ranging from 0 to daily symptoms in IP and/or MP. (c) Most patients had a relative low number of reactions. Thus, the majority of reactions corresponded to a minority of patients, especially in MP.

91.3% of reactions affected a single organ. Multisystemic reactions occurred in 37 patients (1/130 doses), being generally not severe. The type of reactions according to severity and symptoms are represented in Fig 2a and b. No relevant changes occurred in reactions severity between IP and MP. Grade 4 reactions were the most frequent, followed by grades 1 and 2. Isolated mild asthma, the symptom most frequently detected, gathered 76% of reactions classified as grade 4. One grade 5 reaction was recorded. It corresponded to a teenager presenting with hypotension, severe asthma and loss of consciousness during IP due to a wrong dose increase at home after 3 days off treatment. His family decided to continue OIT, without further incidences. Salbutamol was administered in 54 children (1/60 doses) and epinephrine in 9 (1/1467 doses). Thirty-two per cent of reactions during IP coincided with dose increases, and 12% of reactions occurred in the setting of cofactors, affecting 47 children (58%). Cofactors identified (and number of children affected) were post-intake lying down (12), exercise (12), infection (11), emotional stress (8), asthma exacerbation (6), fasting (6), tiredness (5), using straw (1) and NSAID intake (1). Fifty-five patients (67.9%) had had NSAIDs 6 hours before or after CM

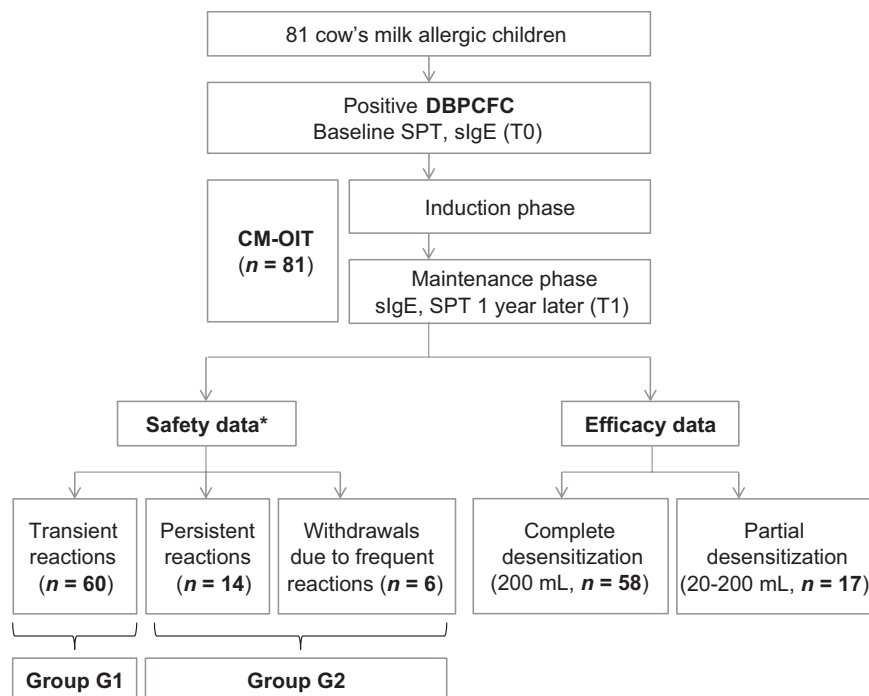


Fig. 1. Study flow chart. CM-OIT, cow's milk oral immunotherapy; SPT, skin prick test; sIgE, specific immunoglobulin E; T0, at baseline; T1, 1 year after CM-OIT start. *The patient treated with omalizumab was excluded from safety analysis, as anti-IgE antibodies might modify CM-OIT safety.

Table 1. Descriptive parameters in the study group ($n = 81$)

Descriptive data	Percentage, mean (SD)* or median (range)†, $n = 81$
Before CM-OIT	
Gender (male)	61.70%
Age (years)**	9.6 (2.9)
Atopic dermatitis	48.0%
Other food allergies	59.0%
Asthma	56.8%
Severity of asthma‡	
Infrequent episodic	24.7%
Frequent episodic	23.5%
Persistent moderate	8.6%
Casein-sIgE TO (KU L^{-1})†	28.3 (0.13–1856)
Cow's milk sIgE TO (KU L^{-1})†	31.1 (0.44–1142)
Casein-SPT TO (mm)*	9.7 (4)
Cow's milk SPT TO (mm)*	11.5 (3.6)
Threshold dose at DBPCFC (mL)†	5 (0.2–150)
Severity of reaction at DBPCFC¶	
Grade 1	3.7%
Grade 2	25.9%
Grade 3	35.8%
Grade 4	34.6%

*Mean (standard deviation), for normal parameters.

†Median (range), for not normal parameters.

‡Severity of asthma according to GEMA guideline [31].

¶Severity at DBPCFC according to Sampson's Grading [33].

**Age distribution at inclusion: 5–6 years: 1 case; 6–8 years: 27 cases; 8–10 years: 27 cases; 10–12 years: 12 cases; 12–14 years: 7 cases; 14–16 years: 3 cases; 16–18 years: 4 cases.

No significant differences were found between severity of reaction at DBPCFC and grading of the most severe previous reaction reported (data not shown).

DBPCFC, double-blind, placebo-controlled food challenge; sIgE, specific immunoglobulin E; SPT, skin prick test; TO, at baseline; T1, 1 year after CM-OIT start.

dose without incidences, nad 62% of lying down-related reactions consisted of asthma symptoms.

The girl treated with omalizumab developed very few and mild CM-dose-related reactions during CM-OIT, despite a pre-omalizumab history of very frequent moderate/severe reactions to CM traces (follow-up: 10.5 months).

Comparison of safety data among groups with transient (G1) vs. persistent (G2) reactions

The frequency of reactions in groups G1 and G2 was strikingly different, as shown in Fig. 3, which reinforces the above-mentioned heterogeneity about reactions' number, distribution and evolution. A higher proportion of grade 4 reactions per child was found in G2 compared with G1 (37% vs. 27% in IP, $P = 0.048$; 33% vs. 0% in MP; $P = 0.044$). Thus, children of G2 ($n = 20$) had frequent and more severe reactions, which tended to slightly decrease over time only in 5 patients. These

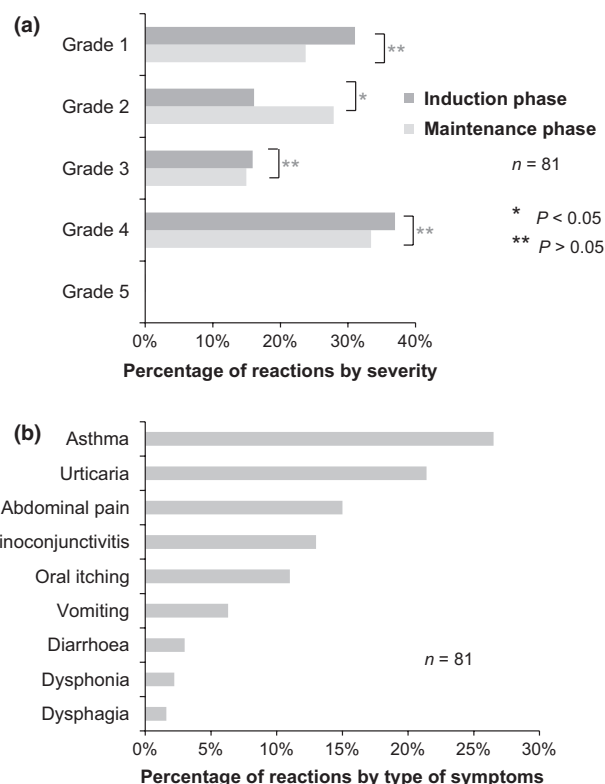


Fig. 2. (a) Reactions distribution according to severity in induction phase (IP) and maintenance phase. (b) Reactions distribution according to type of symptoms.

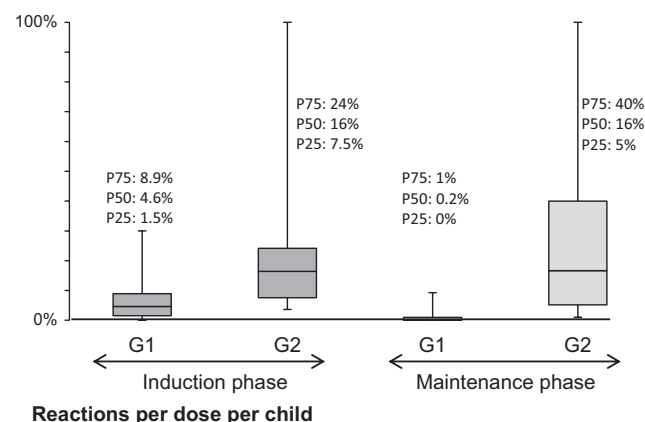


Fig. 3. Reactions per dose per child in IP and maintenance phase (MP) in the group with occasional and transient reactions (G1) and in the group with frequent and persistent reactions (G2). Differences were statistically significant ($P < 0.05$) when comparing G1 vs. G2 both in IP and MP, as well as when comparing G1 in IP vs. MP.

20 patients gathered 78% of all reactions detected. Co-factors justified only 3% of their reactions ($P = 0.032$, G1 vs. G2) and 5 children withdrew CM-OIT due to repeated reactions and milk aversion. One patient was diagnosed with eosinophilic oesophagitis by biopsy because of repeated vomiting and pyrosis after 3 weeks

of CM-OIT and also abandoned. Only 6 of patients in G2 (30%) had achieved complete desensitization ($P < 0.001$, G1 vs. G2), whereas 10 (50%), partial desensitization. Four children required permanent CM-dose reduction due to repeated reactions in MP. When comparing withdrawals with patients in G2 who continued CM-OIT, no differences in reactions frequency/severity in IP nor in clinical/immunological parameters were found (data not shown). Therefore, withdrawals and children with persistent reactions were considered clinically a relative homogeneous group (G2). In contrast, 86.6% of children in G1 had reached complete desensitization (200 mL). Thirty-four per cent of reactions in this group were associated with cofactors, and they could be prevented to some extent afterwards.

Clinical and immunological descriptive data of groups G1 and G2 are compared in Table 2, showing the factors associated with persistent, more frequent and severe reactions.

Both baseline CM-sIgE and casein-sIgE (but neither threshold dose at DBPCFC nor SPT) show a moderate linear correlation with grade 4 and total reactions in IP and MP (Rho Spearman: 0.42–0.59; $P < 0.001$).

Table 2. Comparison of clinical and immunological data in groups G1 and G2.

Factors	Group G1 (n = 60)	Group G2 (n = 20)	Comparison G1 vs. G2
	Percentage or median (range)		P
Asthma	50%	75%	0.037
Severity of asthma [‡]			0.006
Infrequent episodic	26.7%	20%	
Frequent episodic	21.7%	30%	
Moderate persistent	1.7%	30%	
Threshold dose at DBPCFC (mL)	5 (0.6–145)	2.5 (0.2–60)	0.005
Severity of reaction at DBPCFC [¶]			0.027
Grade 1	5%	0%	
Grade 2	28.3%	20%	
Grade 3	41.7%	20%	
Grade 4	25%	60%	
Casein-SPT T0 (mm)*	9 (0.5)	11.9 (4.2)	0.006
Cow's milk SPT T0 (mm)*	11 (3.5)	13.3 (3.6)	0.018
Casein-sIgE T0 (KU L ⁻¹) [†]	15 (0.1–627)	100 (4–1856)	<0.001
Cow's milk sIgE T0 (KU L ⁻¹) [†]	16.6 (0.4–928)	120.9 (7–1142)	<0.001

*Mean (standard deviation), normal parameter.

[†]Median (range), not normal parameter.

[‡]Severity of asthma according to GEMA guideline [31].

[¶]Severity at DBPCFC according to Sampson's Grading [33].

DBPCFC, double-blind, placebo-controlled food challenge; sIgE, specific immunoglobulin E; SPT, skin prick test; T0, at inclusion; T1, 1 year after CM-OIT start.

Interestingly, regardless of the safety profile during CM-OIT, sIgE and SPT decreased after daily exposure to CM throughout 1 year in both groups (Fig. 4). The time required to achieve the maximum-tolerated dose (IP) was not significantly different in G1 (4 months) vs. G2 (5.2 months, $P = 0.194$).

Identification of predictors of persistent reactions under OIT-CM based on survival analysis results

Kaplan–Meier estimate of reactions resolution for the entire group is shown in Fig. 5. The cumulative proba-

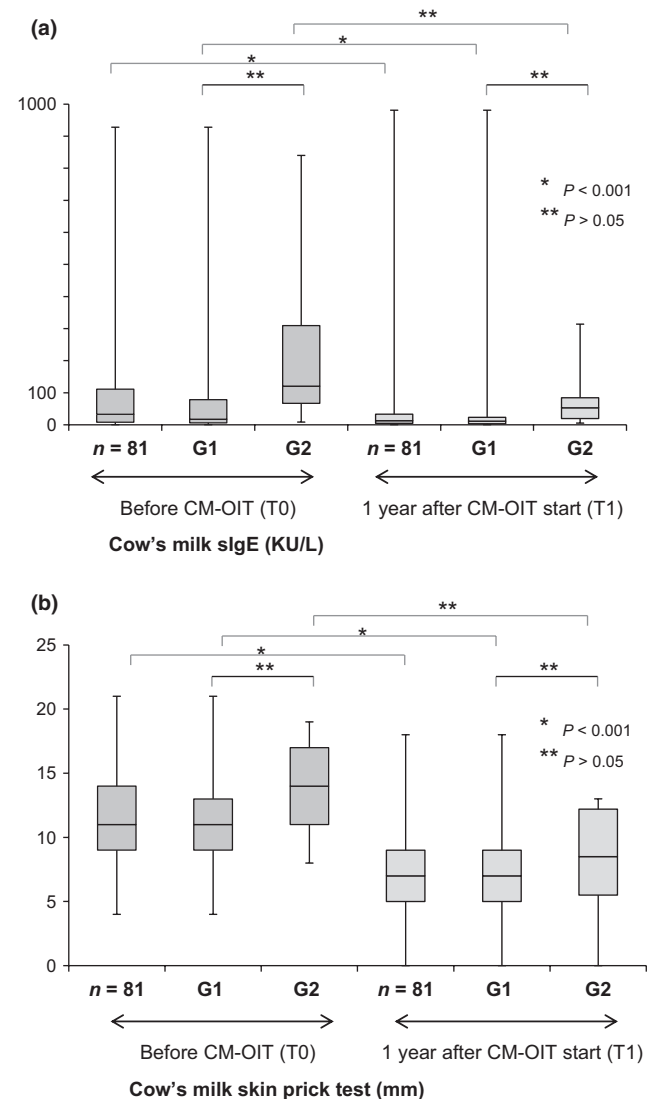


Fig. 4. Cow's milk IgE (CM-sIgE) (a) and cow's milk skin prick test (CM-SPT) (b) levels at baseline (T0) and 1 year after CM-OIT start (T1) in the whole sample ($n = 81$), in the group with occasional and transient reactions (G1) and in the group with frequent and persistent reactions (G2). Differences were statistically significant ($P < 0.05$) when comparing T0 vs. T1 in each group, as well as when comparing G1 vs. G2 both in T0 and T1.

bility of overcoming reactions was 25% after 3 months of CM-OIT (95% CI: 1.9–4.1), 50% (95% CI: 6.1–9.9) after 8 months and 75% after 18 months (95% CI: 7.4–28.6). Both in stratified Kaplan–Meier analysis and univariate Cox proportional hazards regression model, baseline CM-sIgE, CM-SPT, casein-sIgE, casein-SPT and reaction severity grades 2, 3 and 4 at DBPCFC were significantly associated with the resolution of adverse reactions during CM-OIT (Table 3). Reactions resolution was not influenced by age, gender, threshold CM dose at DBPCFC, sIgE modification after 1 year of CM-OIT, pre-existing asthma or its severity, atopic dermatitis or other food allergies. Subsequently, different cut-off points of CM-sIgE (based on definition of sIgE classes I–VI) and CM-SPT were investigated through Cox regression model. The multivariate Cox proportional hazards regression model revealed as independent risk factors for reactions persistence: CM-sIgE classes V–VI [hazard ratio (HR): 2.59; 95% CI: 1.4–4.78; $P = 0.002$], CM-SPT ≥ 9 mm (HR: 2.04; 95% CI: 1.04–4.02; $P = 0.04$) and reaction severity grades 2, 3 and 4 at DBPCFC with respective HR: 12.05 (95% CI: 2.86–52.6; $P = 0.001$), 10.64 (95% CI: 2.5–45.45; $P = 0.001$) and 18 (4.25–83.3). All children in our series fulfilled at least one risk factor. Therefore, the impact of the association of several factors was studied. Through univariate Cox regression model, the association of 2 or 3 of these factors resulted in an increased probability of

reactions persistence with HR: 2.257 (95% CI: 1.14–4.46; $P = 0.019$) and 6.06 (95% CI: 2.7–13.7; $P < 0.001$), respectively.

Discussion

Our study describes thoroughly evolution, frequency, severity and relation with cofactors of all dose-related reactions detected during induction and maintenance phase of CM-OIT following the Spanish-approved SEI-CAP protocol. We recruited throughout 5 years CM-allergic children aged ≥ 5 years for CM-OIT, based on the lower probability of developing natural tolerance beyond this age [4, 5]. Our sample group had a relative high proportion of severe cases, since none was excluded in account of severity of CM allergy, in contrast to some previous studies [16, 17, 19, 25]. Thus, a wide and representative sample of 81 CM-allergic children was followed up for a long period. Most of previous series included smaller samples [12–26] and did not address long-term safety specifically, excluding Barbi's [27] and Meglio's [15]. Indeed, ours is the second-largest series on CM-OIT reported to date, after Barbi's [27]. Moreover, for the first time in literature, we analysed children's tolerance towards CM-OIT on a survival analysis basis, in which the parameter of interest was the resolution of the reactions to CM during desensitization. Predictive factors have been studied to help clinicians identify high-risk patients before starting CM-OIT. Accordingly, this is the first study providing statistical evidence and risk estimation of clinical and immunological parameters related to CM-OIT safety.

The results comparison with previous studies on CM-OIT is hindered by wide methodological differences. In Tables 1 and 2 in the online repository we provide a summary of efficacy and safety results, as well as the main methodological singularities of published series of CM-OIT.

In some aspects, our safety outcomes are concordant with previous reports [12–27]. In previous studies reactions affected 30%–100% of children, tended to decrease over time and were generally mild [21, 25, 29, 35, 36]. Similarly, in our series, reactions affected 95% of children during CM-OIT, but the majority of reactions corresponded to a minority of patients. Moreover, reactions ceased within the first 3–12 months in many cases and multisystemic reactions were infrequent. However, previous reports had already alerted clinicians to adverse events persistence in some cases [27, 28] or when coinciding with particular cofactors [17, 23]. Furthermore, moderate reactions affected 11%–47% of children and one life-threatening event had been reported [37]. Interestingly, based on our unbiased survival analysis, we identified two groups of patients, named group G1 and G2, which corresponded to two

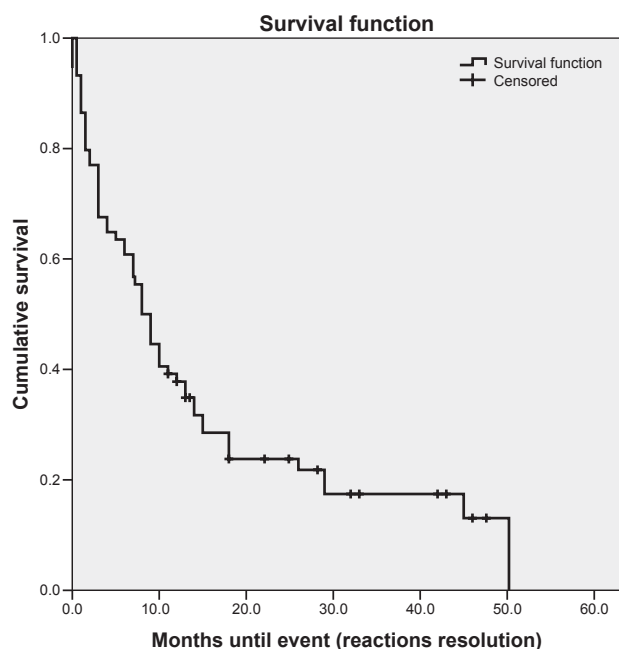


Fig. 5. Kaplan–Meier estimate of survival (resolution of reactions to cow's milk intake). $n = 74$. Regarding the different follow-up time in withdrawals, these six patients were excluded from survival analysis. The patient treated with omalizumab was also excluded from survival analysis, as anti-IgE antibodies might modify CM-OIT safety.

Table 3. Stratified Kaplan–Meier analysis and univariate Cox proportional hazards regression analysis to assess differences in reactions resolution by clinical and immunological variables

Variable	Univariate Cox regression analysis			Stratified Kaplan–Meier analysis		
	Hazard ratio	95% CI	<i>P</i>	Cumulative survival* (months)		
				50th Percentile	95% CI	Log rank (<i>P</i>)
CM-sIgE (KU L ⁻¹)	1.005	1.002–1.009	0.004	NA	NA	NA
CM-sIgE classes V–VI (≥ 50 KU L ⁻¹)	2.967	1.672–5.263	< 0.001	18	4–31.9	< 0.001
CM-sIgE classes I–IV (< 50 KU L ⁻¹)	1	–	–	6	2.3–9.6	< 0.001
CM prick test (mm)	1.135	1.044–1.233	0.003	NA	NA	NA
CM prick test ≥ 9 mm	1.904	1.004–3.61	0.049	9	0.5–5.5	0.016
CM prick test < 9 mm	1	–	–	3	5.2–12.8	0.016
Casein-sIgE (KU L ⁻¹)	1.005	1.002–1.009	0.002	NA	NA	NA
Casein-sIgE classes V–VI (≥ 50 KU L ⁻¹)	2.84	1.618–5	< 0.001	14	3.1–7.9	< 0.001
Casein-sIgE classes I–IV (< 50 KU L ⁻¹)	1	–	–	6	1.6–2.9	< 0.001
Casein prick test (mm)	1.07	1.003–1.144	0.039	NA	NA	NA
Reaction severity at DBPCFC						
Grade 4	27.02	6.25–111.1	< 0.001	10	0–20.3	< 0.001 [†]
Grade 3	14.925	3.57–62.5	< 0.001	8	6.2–9.8	< 0.001 [†]
Grade 2	12.82	3.03–52–63	0.001	6	1.8–10.2	< 0.001 [†]
Grade 1	1	–	–	0.5	0–1.3	< 0.001 [†]

*Cumulative survival refers to the time until reactions resolution.

[†]Stratified log rank: *P* < 0.001 for grade 1 vs. grades 2, 3 and 4; *P* = 0.046 for grade 4 vs. grades 3 and 2; *P* = 0.541 for grade 2 vs. grade 3.

95% CI, 95% confidence interval; DBPCFC, double-blind, placebo-controlled food challenge; NA, not applicable; sIgE, specific immunoglobulin E.

clear clinical patterns in the outcome of CM-OIT. Group 1 included 60 patients (75% of all treated patients), who developed reactions only occasionally, mainly triggered by known cofactors and which ended to resolve over time. In them, our protocol could be considered safe and efficacious. On the other extreme, group 2 included 20 patients (25% of all treated children) and their reactions were frequent, more severe and mainly unpredictable, persisting over time or leading to withdrawal. In them, CM-OIT was insufficiently safe. Thus, as a main achievement of our work, we have identified and characterized a high-risk group of patients during CM-OIT.

Cofactors played a role in many children (58%) and, therefore, should be taken into account. It is noteworthy that lying down as cofactor has not been previously reported. We hypothesize that silent gastroesophageal reflux and subsequent milk inhalation might be the underlying mechanism of lying down associated asthma. NSAIDs seem to act as a cofactor only in particular cases. A life-threatening reaction occurred coinciding with a wrong dose increase after 3 days of treatment, highlighting that strict adherence to CM-OIT is mandatory.

Based on the defined clinical patterns observed during OIT-CM, we aimed at identifying predictive properties of clinical and immunological features that would help us identify, prior to desensitization, those children in which CM-OIT might be insufficiently safe. In our study, sIgE correlated moderately with the number and severity of reactions during CM-OIT. Further-

more, baseline CM-sIgE ≥ 50 KU L⁻¹, CM-SPT ≥ 9 mm and reaction severity grades 2.3 and 4 at DBPCFC resulted independent risk factors of reactions persistence. The association of two or three of the above-mentioned factors defined patients at high risk for persistent reactions during CM-OIT, with hazard ratios of 2.26 and 6.06, respectively. All patients in G2 fulfilled at least two of these criteria. Other authors found difficulties in CM-OIT of patients with high sIgE [13, 14, 17, 23, 26] and asthma/rhinoconjunctivitis [23] providing, however, no statistical evidence or risk estimation. In our series, pre-existing asthma was associated with the group with persistent reactions, but did not influence survival function.

The factors associated in literature with CM allergy severity and persistence (higher CM-sIgE and SPT, coexisting asthma/rhinitis, smaller eliciting CM-dose) [2, 6, 7] coincide with those related to the failure and highest risk in CM-OIT [9]. As patients with a more persistent and severe profile would be the most benefited if desensitized, they should not be excluded theoretically from OIT procedures [8, 26]. Nonetheless, further strategies should be investigated to improve safety in those high-risk patients, such as our group G2. In this sense, Barbi describes a series of 132 severe cases under CM-OIT during 24 months, in which premedication (antihistamines and chromones), a slower schedule and a longer in-hospital rush phase were used [27]. He reported a lower rate of adverse events and a higher proportion of predictable reactions. Thus, the suitability of our protocol for high-risk cases should be analysed.

In our work, the time to achieve the maximum-tolerated dose was not significantly different in children with or without frequent and persistent reactions. We used no premedication, in contrast to other groups [13–15, 17, 22, 25, 26]. Similar to Barbi's and Longo's, our CM-OIT scheme included a first in-hospital rush phase, in contrast to most schedules [14, 16, 18, 21, 23]. It is likely that children in G2 might have benefited from a more progressive increase schedule and/or premedication. Accordingly, we suggest that slower schedules and premedication might be considered in patients who fulfil the above-mentioned high-risk criteria. Unfortunately, evidence of the impact of premedication on CM-OIT safety and efficacy is lacking [9]. Omalizumab would seem to be an excellent tool in extremely reactive patients, as shown in one girl in our study. However, data about treatment duration and allergen tolerance after omalizumab interruption are insufficient [22, 38]. Recently, sublingual immunotherapy for milk allergy has also been proposed with promising safety results [21]. Furthermore, hypoallergenic forms of food, such as extensively heated ovomucoid for egg allergy, have shown a better safety profile than the native allergen in preliminary studies in mice, being equally effective to induce desensitization [39].

Limitations

Due to the absence of a control group, we were not able to determine to which extent the acquisition of natural tolerance beyond the age of 5 years could have influenced our long-term efficacy and safety results. Our groups G1 and G2 might intrinsically differ in their capacity to develop natural tolerance, regarding to the above-mentioned factors associated to CM allergy persistence. Indeed, our group G1 would correspond to a better prognostic profile and, thus, spontaneous recovery could justify some of our positive results in this group. Furthermore, it could be hypothesized that CM-OIT might have only accelerated natural tolerance in this group of good prognosis. Classically, it has been stated that IgE-mediated CM allergy is outgrown in 63%–75% of cases within the first 3–5 years of life, being later development of tolerance less probable [2–5]. Accordingly, we selected for CM-OIT children with persistent CM allergy older than 5 years. Nevertheless, more recent studies reported later achievement of natural tolerance in 21% of cases between ages 5 and 10 years in a Portuguese referral cohort of 66 children [7] and in 56% of cases between ages 6 and 18 years in Skripak's American referral cohort of 807 children [6]. If we extrapolate to our study population Skripak's data about the incidence of tolerance acquisition for each 2-year interval between ages 6 and 18 years, only 10% of our children would have achieved natural tolerance

within our median follow-up period of 25 months. Therefore, we consider that our satisfactory safety and efficacy results in 75% of our population could be barely explained alone by the achievement of natural tolerance during follow-up. Furthermore, although up to 56% of our patients might have developed spontaneous tolerance along childhood and adolescence, CM-OIT would have permitted, at least, an earlier diet diversification and accidental reactions prevention several years before oral tolerance in 65% of them. Moreover, desensitization would have been achieved in an additional 19% of children in which tolerance had not been expected.

Our research about safety of CM-OIT was performed as an open – not blinded – study, due to the strong familiar commitment required for CM-OIT. However, we tried to minimize the impact of a potential subjective component in reports by considering only objective allergic signs closely related to CM intake.

Conclusions

CM-OIT is an experimental therapeutic approach with proven ability to induce desensitization, but safety is still concerning. In our study, reactions to CM intake were heterogeneously distributed: in 60 children (75%), reactions were infrequent, mild–moderate, ceased over time and were frequently induced by known cofactors. In these children, CM-OIT could be considered safe. Instead, 20 children (25%) had frequent, more severe and generally unpredictable reactions, which persisted over time or led to withdrawal. CM-sIgE ≥ 50 KU L⁻¹, CM-SPT ≥ 9 mm and reaction severity grades 2, 3 and 4 at DBPCFC were independent predictors of reactions persistence. The association of ≥ 2 of these clinical and immunological parameters might help clinicians identify high-risk patients before CM-OIT. In these particular patients, who correspond to those with the most severe and likely permanent CM allergy phenotype, individualized CM-OIT schedules and premedication should be considered to minimize adverse events.

Our results reinforce that before CM-OIT can be recommended for routine practice, further evidence of the impact of CM-OIT schedules, administration routes, premedication and allergen modification on safety and efficacy should be provided through large randomized controlled trials.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Methodological features, efficacy and safety-related data of published studies on CM-OIT.

Table S2. Main methodological differences of previous studies on CM-OIT which could influence outcomes and hinder comparisons among studies.

Table 1.

Methodological features, efficacy and safety-related data of published studies on CM-OIT.

Parameter	Previous studies on cow's milk oral immunotherapy															
	Patriarca ^[12]	Patriarca ^[13]	Meglio ^[13,14]	Staden ^[23]	Staden ^[24]	Morisset ^[16]	Skipak ^[17]	Narisety ^[25]	Pajno ^[18]	Zapatero ^[19]	Longo ^[26]	Barbi ^[27]	Martorell ^[20]	Keet ^[21]	Nadeau ^[22]	Vazquez
Study design	NC Open	NC Open	NC Open	RCT Open	NC Open	RCT Open	RCT Blind	Follow-up Open	RCT Blind	NC Open	RCT Open	Follow-up Open	RCT Open			NC Open
N (cases in CM-OIT)	29	18	21	25	9	27	13	15	13	18	30	132	30	30	11	81
Age: years. Mean (range)	NM	8,2 (3-12)	6,9 (5-10)	2,5	7 (3-14)	2,2 (1-6,6)	9,3	6-16	9 (4-12)	5 (4-8)	7,9 (5-17)	7,2 (3-20)	2,2 (2-2,9)	6-17	7-17	9,6 (5-17)
Premedication	AH + CG	AH + CG	AH	No	No	No	AH	No	No	No	AH	AH + CG	No	No	OM	No
Follow-up: months. Mean or range	3-12	7-9	65	8	3-7 days	6	5	3-17	4,2	8-12	12	24	14,2	15	5,5	25,4
Induction phase schedule: days	136	177	180	67	3-7	42	56	90-510	126	70	10 +home (NM)	10 +home (NM)	114	140	49-77	114
Desensitization rate: % of Cases, CM Dose in ml	66%, ≥130	83%, ≥130	71%, ≥200	48%, 250	66%, 120	89%, ≥200	92%**	93%**	77%, ≥200	81%, ≥200	36%, ≥150	64%, ≥150	90%, ≥200	90%**	90%, ≥60	71%, ≥200
Reactions: % of Cases	51%	31%	62%	100%	100%	NM	100%	>80%	77%	72%	100%	64%	80%	NM	100%	95%
Mild* R: % of Cases	100%	Most	43%	84%	89%	33%	90% of R	>18% of D	53%	55%	100%	22- 64%	33%	24% of D	1%	83%
Moderate* R: % of Cases	0	NM	14% (A)	16%	11% (A)	50%	8%of D(A)	NM	23%	16%	40-46%	NM(>21%)	47%	0,6%of D	0,3%	69%
Multisystemic reactions	NM	NM	14% of R	NM	11% of R	NM	1% of D	6% of D	38% of C	16% of C	NM	NM	37% of C	0,4%of D	NM	11% of R
Withdrawals due to R (% of C)	17%	16%	35%	36%	0%	11%	8%	0%	15%	11%	10%	12%	3%	6,6%	10%	7%

Abbreviations. A: asthma; AH: antihistamines; C: cases; CM-OIT: cow's milk oral immunotherapy; CG: chromoglycate; D: doses; IP: induction phase; NM: not mentioned; OM: omalizumab; R: reactions. *Mild and moderate reactions: when the definition of severity was not specified in the article, reactions described were classified according to Sampson's Classification as mild if grade 1-3 or moderate if grades 4-5 [33]. CG was used for 2-4 weeks if mild symptoms/abdominal pain. AH were used until 2-4 weeks after end dose. [12,13,26]. ** Efficacy was defined as increase in threshold dose at food challenge [17,25,21].

Table 2. Main methodological differences of previous studies on CM-OIT which could influence outcomes and hinder comparisons among studies.

Previous studies on CM-OIT	Main methodological singularities
Patriarca ^[12]	Included 47 adults and children desensitized to different foods. Reactions reported for the total group. Premedication was used (AH, CG).
Patriarca ^[13]	Included 36 children desensitized to different foods. Asthma was considered a mild reaction. Premedication was used (AH, CG).
Meglio ^[13,14]	Included no CM/casein-sIgE >100 KU/L. Premedication was used (AH).
Staden ^[23]	Included egg and CM allergy. Young children (mean age: 2,5 years).
Staden ^[24]	Included no CM-sIgE >100KU/L. In-hospital protocol.
Morisset ^[16]	Included only CM allergic children who tolerated ≥ 60 ml at baseline. 26% had non-IgE mediated CMA. Young children (mean age: 2,2 years).
Skripak ^[17]	Excluded severe CMA. No CM-sIgE > 100KU/L. Efficacy was defined as increase in threshold dose at DBPCFC. Premedication was used (AH).
Narisety ^[25]	Corresponded to follow-up of Skripak's study. Individualised increase for 3-17 months was performed.
Pajno ^[18]	Weekly (not daily) increase and dosage at hospital.
Zapatero ^[19]	Only 11% had CM-sIgE >10 KU/L (range: 0,35- 14).
Longo ^[26]	Included only severe CMA. In-hospital rush phase (10 days) followed by personalized slow increase at home. Premedication was used (AH).
Barbi ^[27]	Included severe CMA. Longo's protocol. Only in-home reactions reported, after rush phase. Obviated mild skin and digestive symptoms. Particular rescue treatment.
Martorell ^[20]	Included no CM-sIgE >50 KU/L. . Young children (mean age: 2,2 years). Particular classification of reactions (asthma considered mild).

Keet ^[21] Efficacy end point: at least 10-fold increase in threshold dose at DBPCFC after 60 weeks in maintenance phase.
Compared SLIT (N=10) vs OIT (N=20).

Nadeau ^[22] Highly CM-sensitized children (median sIgE: 50KU/L).
Omalizumab was used from 9 weeks before until 16 after CM-OIT start.

Vazquez Included severe CMA (44% had CM-sIgE >50 KU/L) .
In-hospital rush phase (2-4 days). No premedication.
All reactions during CM-OIT induction and maintenance phases reported.
Severity assessed according to Sampson's Classification^[33].

CM: cow's milk; CMA: cow's milk allergy; OIT: oral immunotherapy; sIgE: specific immunoglobulin E; SLIT: sublingual immunotherapy. DBPCFC: double-blind placebo-controlled food challenge.