ORIGINAL ARTICLE

Cow's milk oral immunotherapy in real-life: up to 15-year follow-up study

Ângela Gaspar[1], Susana Piedade[1], Cláudia Monteiro-Marques[1], Inês Mota[1], Luís Miguel Borrego[1,2]

[1]Department of Immunoallergy, Hospital da Luz Lisboa, Lisbon, Portugal
[2]Comprehensive Health Research Center (CHRC), NOVA Medical School, Universidade Nova de Lisboa, Lisbon, Portugal

Corresponding author:
Ângela Gaspar, MD
Immunoallergy Department, Hospital da Luz Lisboa
Av. Lusíada 100, 1500-650 Lisbon, Portugal
E-mail: angela.gaspar@sapo.pt
Orcid ID: 0000-0001-8330-8016

Manuscript accepted for publication

Summary

Background. Oral immunotherapy (OIT) is a promising strategy for severe and persistent cow's milk (CM) allergy. However, clinical experience concerning long-term follow-up is scarce. The objective is to assess long-term efficacy and safety of maintenance phase of OIT in real-life. **Methods.** Prospective study of children with severe IgE-mediated CM allergy that underwent CM-OIT, and were followed-up by the authors, up to 15 years. Complete desensitisation was defined when maintenance dose of 200 mL daily was achieved. Characterization (clinical and laboratory) was performed before and after OIT during follow-up. **Results.** Thirty-three patients enrolled: 58% male, 70% asthmatics and 67% with previous history of CM-anaphylaxis. Mean age at onset of CM-OIT was 7±2.8 years. Complete desensitisation was ensured in 94% (free diet with dairy 200mL of CM or equivalent). During maintenance phase 79% developed allergic reactions, with anaphylaxis in 33%, including exercise-induced anaphylaxis in 15% and with cheese intake in 15%. We stress that eosinophilic esophagitis appeared in 12%. Specific IgE levels and skin prick tests (SPT) to CM and casein have significantly decreased, and in most patients (61%) SPT became negative. When available, specific IgG4 levels have significantly increased. **Conclusions.** This real-life study supports long-term efficacy and safety of CM-OIT. CM-OIT had a high success rate, allowing diet without restrictions with persistence of the effect up to 15 years. However, one-third of patients experienced anaphylaxis during maintenance phase. CM-OIT should always be performed by allergy experts and only motivated families should be enrolled, since long-term follow-up is required, and daily intake of CM is needed to ensure desensitization.

Key words
Cow's milk allergy; desensitization; food allergy; Long-term follow-up; oral immunotherapy.

Impact Statement

Real-life study that supports efficacy and safety of cow's-milk-oral-immunotherapy in long-term follow-up, with high success rate of complete desensitization, although one-third had anaphylaxis during maintenance-phase and some had eosinophilic esophagitis.

INTRODUCTION

Cow's milk allergy (CMA) is the most common food allergy in children worldwide and can affect up to 3 to 5% of children in the first years of life (1-4). In our country, cow's milk (CM) is also the food most frequently responsible for cases of food allergy in paediatric age (5,6), as well as being the main cause of anaphylaxis and the leading cause of recurrence of anaphylaxis in children (7,8).

CM is a hidden allergen, widely disseminated in processed foods, which is why CMA is a disease associated with a marked reduction in the quality of life of the patient and their family, negative psychological and social impact, as well as a risk to life, particularly in severe forms of the disease (2,8).

Most children outgrown CMA in early childhood, by five years of age (3,6). However, CMA is increasingly persistent, with more than a third of cases lasting into adolescence (9,10). In cases mediated by immunoglobulin E (IgE), the prognosis is worse as duration of the disease increases (9,10). This reality becomes even more worrying and causes marked anxiety in patients and their families (11,12) when CMA is severe and the probability of accidental contact with the hidden allergen is high. Risk factors for persistence of CMA have been identified: existence of asthma (9,13) and higher levels of serum CM specific IgE; greater persistence associated with values ≥ 5 kU/L and worse prognosis if ≥ 20 kU/L (9), although values vary between the populations studied (13,14).

With the enormous variety of processed food products, a strict avoidance diet of cow's milk proteins (CMP) is difficult to implement and is associated with a high risk of accidental ingestion, after trace amounts exposure of CMP, with consequent allergic reactions, which can be serious or even fatal (10,15,16). In a twenty-year study conducted in the United Kingdom, CM was identified as the main cause of fatal anaphylaxis in school-age children, accounting for a quarter of all cases of death from anaphylaxis in school-age children (15). Currently, CM is globally considered the main cause of fatal anaphylaxis in school-aged children, with the highest risk occurring in adolescence (10,15,17).

In cases of persistent and severe CMA, the classic therapeutic approach, based on an elimination diet, is insufficient and associated with a high risk of severe allergic reactions due to inadvertent ingestion of CMP. Oral immunotherapy (OIT), an innovative strategy, has been recognized as promising, effective and reasonably safe treatment for persistent CMA (6,10,12,18,19). It is the only therapeutic strategy capable of modifying the natural history of the disease, providing protection against inadvertent ingestion and thus improving the quality of life of the patient and their family.

There are several published CM-OIT protocols with varied approaches (oral, sublingual, sublingual-oral), diverse escalation doses schemes, and durations, until maintenance phase. Overall, CM-OIT protocols include two distinct stages: a build-up phase (between the first dose of CM, usually a very low dose, with progressive increases of threshold doses, up to the target dose) and a maintenance phase, in which the patient ingests the defined target dose of CM daily for years. The target dose of 200mL is the dose accepted by consensus as the criterion for success of OIT by most authors (20-27).

The main goal of CM-OIT is to achieve a state of clinical tolerance, which is defined as the ability to consume CM without developing allergic symptoms. Nevertheless, what happens in CM-OIT is a state of desensitisation, which is defined as the ability to increase the threshold of tolerance to a dose amount of milk without symptoms while the patient is on the given therapy. Desensitisation is dependent on regular milk intake and may be lost if regular intake of CMP is interrupted. Thus, in most studies, it is defined by the ability to tolerate daily intake of a normal amount of milk or dairy products (usually 200mL, corresponding from 6.4 to 7g of CMP), reacting again if regular intake is suspended. Therefore, once the maintenance phase is achieved, the patients on CM-OIT are advised to keep regular intake of CM to ensure the desensitisation.

The ultimate goal of CM-OIT, the state of remission or sustained unresponsiveness (10,12,19) may not be achieved in many patients (18,28,29). It is defined as a permanent absence of clinical reactivity to milk even if it is not consumed regularly and persists even after stopping treatment

Manuscript accepted for publication

for a period of time. It is long-lasting and persistent beyond CM-OIT, even in the absence of regular intake of CMP.

Clinical experience concerning long-term follow-up of patients who underwent CM-OIT is scarce. In this survey, we aimed to assess the real-life long-term efficacy and safety of the maintenance phase of a CM-OIT protocol that the authors have been using for up to 15 years.

The authors present children and adolescents with IgE-mediated CMA who underwent specific CM-OIT and have been under a long-term follow-up by the authors up to 15 years. We aimed to assess the long-term efficacy and safety of CM-OIT in these patients, and to analyse their clinical (milk consumption, clinical symptoms), skin prick tests (SPT) and laboratory evolution during the described years of follow-up. Adherence to the protocol, defined as daily consumption of CM and free diet, along with the occurrence of allergic reactions during the maintenance phase were evaluated. Furthermore, we sought to examine the course of maintenance phase in high-risk patients, namely those with asthma and a previous history of CM-anaphylaxis.

MATERIALS AND METHODS

Study design

We have performed a prospective and non-controlled study of children and adolescents who underwent CM-OIT and were subsequently followed at our Immunoallergy department on maintenance dose (200mL CM daily) after reaching the maintenance phase.

Patients

Children and adolescents, with persistent and severe IgE-mediated CMA diagnosis were considered eligible for CM-OIT. CMA diagnosis was based on clinical history, and an IgE-mediated underneath mechanism was confirmed in all patients, by the presence of positive SPT and positive specific IgE (14,30). Before starting CM-OIT all children had a positive CM oral challenge or had a reproducible history on the previous year of an allergic reaction after exposure to small amounts of CM (30).

Methodology

SPT to CM and milk proteins (casein, beta-lactoglobulin and alpha-lactalbumin) were performed before and after OIT protocol with allergen extracts (Roxall, Hamburg, Germany). As positive control, a histamine solution at a 10mg/mL concentration was used and as a negative control, a saline solution was used. SPT results were read after 15 minutes, and a mean wheal diameter of $\geq$ 3 mm was considered as a positive cut-off.

Serum levels of specific IgE (ImmunoCAP®, Thermo Fisher Scientific, Waltham, Massachusetts, USA) to CM and milk proteins (casein, beta-lactoglobulin and alpha-lactalbumin) were also performed before and after protocol. Specific IgE was considered positive if $\geq$ 0.35kU/L. When available, specific IgG4 levels to CM and casein were performed before and after OIT protocol.

After OIT protocol the patients also performed SPT to goat's milk allergen extract (Leti, Madrid, Spain) and specific IgE to goat's milk and sheep's milk. If necessary, SPT to fresh sheep's milk (prick-to-prick) was also made.

The eligibility criteria for CM-OIT were in accordance with the guidelines (**Table I**) (2,6,10,18,30). The indications and absence of contraindications were confirmed in all patients.

In patients with gastroesophageal reflux or recurrent digestive symptoms, upper digestive endoscopy with esophageal biopsies and eosinophil counts where always performed before starting the protocol, to rule out eosinophilic esophagitis, which is an absolute contraindication for CM-OIT.

Coexisting respiratory and cutaneous allergic diseases (asthma, allergic rhinitis and atopic dermatitis) were properly controlled before the beginning of the CM-OIT protocol and were kept under surveillance during the maintenance phase.

Anaphylaxis was defined according to clinical criteria of the European Academy of Allergy and Clinical Immunology (EAACI) (31).

Manuscript accepted for publication

A trained medical team experienced in CM-OIT performed the overall clinical evaluation of all patients. Additionally, a systematic laboratory plan for follow-up was settled during the maintenance phase of the CM-OIT protocol.

CM-OIT protocol

The protocol used is presented in **Table II** (23). It consisted of a mixed sublingual and oral protocol using UHT (ultra-high temperature) pasteurized CM. The build-up phase involved the administration of progressively increasing amounts of whole, non-diluted, unheated CM, starting with a sublingual dose of one drop, followed by gradual titration at predefined intervals. The initial doses were administered sublingually (contact with the oral mucosa from two to five minutes, followed by expulsion of the allergen), and subsequently transitioned to oral ingestion of increasing doses up to a target dose of 200mL per day. Up-dosing was always performed in the day hospital setting. During each hospital session, a fixed daily home dose was established for continued ingestion between visits. The CM-OIT protocol typically included five sessions (adjustable in cases requiring slower dose escalation) at variable intervals, over an average period of 12 weeks. During the build-up phase, additional sublingual or oral doses could be administered, if clinically indicated, particularly in the presence of local or systemic reactions. The visit schedule was flexible, depending on the response to treatment and the convenience of the patient and their caregivers, with additional visits and dose adjustments when necessary. Between sessions, patients continued the last tolerated dose at home, always after meals and avoiding vigorous physical exercise for the following two hours. The same recommendations applied during the maintenance dose.

During the maintenance phase, follow-up visits were scheduled at one month, six months, and one year after reaching the maintenance dose, and subsequently on an annual basis or whenever clinically indicated.

A written emergency plan was given to all the patients. All patients and caregivers have been instructed to have always emergency medication available for the management of allergic reactions (10,12,19,31), including: adrenaline auto-injector (AAI), oral non-sedative antihistamine, and oral corticosteroid. For patients with asthma, an inhaled short-acting beta2-agonist bronchodilator was also included. The medical team ensured continuous support, maintaining 24-hour telephone availability to address clinical concerns, provide guidance, and respond to urgent situations.

All patients continued their daily preventive therapies, including oral antihistamine therapy, and performed, when necessary, therapies for coexisting allergic conditions.

Complete desensitisation was defined when the target dose of 200mL daily intake (corresponding to 6.4 to 7g of CMP) was achieved with clinical tolerance (6,10). **Partial desensitisation** was defined if the daily dose achieved was less than 200mL.

The study was approved by the ethics committee, and all procedures were conducted according to the principles of the Declaration of Helsinki. All legal guardians were fully informed about the procedures (risks and possible adverse reactions) using written documents, and written informed consent was obtained for all patients in all hospital sessions of the protocol.

Statistical analysis

Quantitative variables with normal distribution were presented as mean (±standard deviation). Variables without normal distribution were presented as median and range (minimum-maximum). Categorical variables were described as frequencies and were expressed as percentages. The Fisher's exact test was used to test the association between variables, and results were expressed with p-values and odds ratios (OR) with confidence interval of 95% (95%CI). Paired comparisons for pre- and post-OIT observations were performed with Wilcoxon matched-pairs signed rank test, and otherwise group comparisons were performed with the Mann Whitney test. Statistical significance was defined as a p-value <0.05. The analysis was performed using GraphPad Prism version 10.6.0 for Windows, GraphPad Software, Boston, Massachusetts, USA (www.graphpad.com).

Manuscript accepted for publication

RESULTS

Patients

We enrolled 33 patients (**Table III**) who underwent CM-OIT protocol. The mean age at the onset of CM-OIT was 7.0±2.8 years. Most patients were male (58%), with a male-female ratio 1.4:1.
Almost all patients had other allergic diseases (97%) with significant prevalence of asthma (70%). Only one patient did not suffer from another allergic comorbidity.
Before starting CM-OIT all children had proven CMA. Serum specific IgE and SPT to CM and all CMP were both positive in all children (**Table IV**). The mean specific IgE to CM at the onset of CM-OIT was 23.02 (±19.84) kU/L, and the mean wheal diameter to CM extract in SPT at the onset of CM-OIT was 8.92 (±3.85) mm.
We point out that 22 children (67%) had previous history of CM anaphylaxis, and 9 children (27%) had history of 3 or more episodes of CM anaphylaxis. Most of the patients included had, during last year, a reproducible history of an allergic reaction after exposure to small amounts of CMP. In cases where there was no history of accidental contact in the previous year, a CM oral challenge was performed before starting CM-OIT, which was positive in all cases, from only sublingual contact to less than 5mL of CM.

Clinical outcomes

The mean time of follow-up during the maintenance phase was 8.0±4.4 years. Regarding the adherence to CM-OIT protocol during this period, 94% (31 patients) maintained diet without restrictions including daily ingestion of 200mL of CM or equivalent. One patient had partial desensitisation (with daily ingestion of 20mL CM) due to the appearance of eosinophilic esophagitis. One patient completely suspended CM intake due to the appearance of eosinophilic esophagitis.
During the maintenance phase 26 patients (79%) developed allergic reactions, and 19 patients (58%) had 3 or more episodes of allergic reactions. Cutaneous, oropharyngeal, and gastrointestinal symptoms were the most predominantly reported reactions. Most of these allergic reactions occurred in the presence of cofactors, such as infections (n=12), exercise (n=10), cheese intake (n=7), emotional stress (n=5), pollinic season (n=5), menstruation (n=2), fasting (n=2), sun exposure (n=1). We point out that 11 patients (33%) had anaphylaxis during maintenance phase, including exercise-induced anaphylaxis in 5 patients (15%) and anaphylaxis related to cheese intake (sheep or goat milk proteins) in 5 patients (15%).
The occurrence of allergic symptoms, even severe reactions, did not lead to discontinuation of CM-OIT. There was a total of 15 episodes of anaphylaxis during the maintenance phase (one patient had 3 episodes, and two patients had 2 episodes). Most of these anaphylactic reactions occurred in the presence of cofactors, such as exercise (n=6) or during infections (gastroenteritis-2). Regarding the anaphylactic reactions induced by exercise most of them (4 out 6) occurred after 2 years of maintenance phase (in two adolescents occurred at 6 years and at 8 years of maintenance phase). Six anaphylactic episodes were related to accidental ingestion of sheep or goat milk proteins (goat or sheep cheese-5, goat milk-1). Adrenaline was required in 4 of these anaphylactic episodes, with use of AAI in 3 episodes. All episodes had complete resolution after proper treatment (most cases with oral and inhaled medications).

Clinical risk factors

Since 33% of patients had anaphylaxis during the maintenance phase, we have analyzed which clinical factors could be associated with the occurrence of these severe allergic reactions. We have included a significant number of high-risk patients: 70% of asthmatics, 67% with previous history of CM anaphylaxis and 52% with both risk factors (17 patients had asthma and previous history of CM anaphylaxis).
Among patients with previous history of CM anaphylaxis (22 patients), we found that 10 of them had anaphylactic reactions during the maintenance phase. An association between the occurrence of anaphylaxis during maintenance phase and the previous history of CM anaphylaxis seems to

Manuscript accepted for publication

be present (45% of anaphylaxis during the maintenance phase in the group with previous history CM anaphylaxis, comparing to only 9% in those without previous history of CM anaphylaxis), although without statistical relevance (p=0.0543).
Asthma history was more frequent in patients with anaphylactic reactions during the maintenance phase (82%). Nevertheless, the coexistence of asthma was not a risk factor for the occurrence of anaphylaxis during the maintenance phase (39% of anaphylaxis in the group of asthmatics, compared to 20% in those without asthma; p=0.430).

Skin prick tests and laboratory outcomes
The results of serum specific IgE to CM and casein, and SPT to CM and casein, before and after OIT are presented in **Table IV**. Specific IgE levels to CM and to casein have progressively decreased, with a statistically significant difference between prior to and after OIT (p<0.0001, Wilcoxon). **Figure 1A** and **Figure 1B** show the evolution over time of serum levels of specific IgE for CM and casein, respectively, immediately before beginning OIT (T0), after the first year of follow-up (T1) and at the last assessment after OIT (T2). SPT to CM and to casein have also progressively decreased, with a statistically significant difference before and after OIT (p<0.0001, Wilcoxon). In 20 patients (61%) the SPT to CM and casein became negative.
The results of serum specific IgG4 levels to CM and casein before and after OIT are presented in **Table IV**. Only 12 patients performed specific IgG4 levels before OIT. Serum specific IgG4 levels to CM and casein before and after OIT have increased, with statistically significant difference (respectively p=0.0353 and p<0.001, Mann Whitney).
Comparing patients with and without anaphylaxis during maintenance phase (22 and 11 of them, respectively), we found that the specific IgE levels to CM and to casein prior to OIT where higher in the patients with occurrence of anaphylaxis during the maintenance phase, although without statistically significant difference (respectively p=0.0779 and p=0.1045, Mann Whitney). The mean specific IgE levels to CM in the 22 patients with anaphylaxis during maintenance phase was 33.36 (±20.75), compared to 19.09 (±17.54) in the 11 patients without anaphylaxis during maintenance phase. Even if we define a cut-off value of 5 and 20 kU/L for specific IgE levels to CM we found the same result (not statistically significant; respectively p=0.1411 and p=0.2451, Fisher´s test).

DISCUSSION

This real-life study supports long-term efficacy and safety of CM-OIT. Our results have shown a high success rate to this OIT protocol, since complete desensitisation was achieved in 94% of patients. Daily intake of 200mL of CM or equivalent ensured desensitisation and enabled a diet without restrictions. However, during the maintenance phase most patients (79%) developed allergic reactions and one-third (33%) had anaphylaxis, with a total of 15 episodes of anaphylaxis (including six episodes induced by physical exercise and six episodes associated with accidental ingestion of goat and sheep milk proteins; five of them in the context of cheese consumption).
A history of previous CM-anaphylaxis was associated with a higher frequency of occurrence of anaphylaxis during the maintenance phase of OIT. However, a history of severe CMA is not a contraindication for CM-OIT. On the contrary, it is these patients with severe forms of the disease who usually benefit most from CM-OIT. In fact, notably most of our patients (67%) had severe CMA with a history of CM anaphylaxis.
In recent years, there has been various publications on this innovative approach, with several studies confirming high success rates in immunotherapy with CM (20-26,32-34), including controlled and randomised studies (21,22,24,25,29,33-38), as well as the persistence of the effect after several years of OIT (26,29,39,40), generally dependent on maintained daily exposure to CMP.

Manuscript accepted for publication

Most protocols use the oral route and a few, to a lesser extent, the sublingual route. There are many differences between the published protocols, particularly regarding the age of onset and characteristics of the patients included, initial dose, rate of progression, maintenance dose, product used, among others. In most studies, pure natural CM is the product used, undiluted, or diluted at 1:10, 1:25 or 1:100. The use of baked milk has been proposed by some authors because it is less allergenic, as it alters the structure of conformational epitopes (10,41), but it has been shown to be less effective for desensitisation (2,42,43). It should be noted that the World Allergy Organization (WAO) guideline does not recommend OIT with baked milk (2). Similarly, the use of heated milk has also been proposed by some authors for the same reason, but it has also been shown to be less effective for desensitisation (44).

In the published literature, there is variability in success rates, depending on the type of protocol, characteristics of the treated population, target dose considered, and criteria for evaluating results. Nevertheless, most studies showed high desensitisation efficacy frequencies, above 70% (20-26,33,34,36). A meta-analysis that included 469 children, published in 2022 (36), encompassing 11 controlled and randomised studies of oral immunotherapy with CM, showed a significant benefit in terms of desensitisation efficacy (73%) for CMA (OR: 7.35; 95%CI: 2.82-19.13). The benefit was more significant in children over three years of age (OR: 18.05; 95%CI: 6.48-50.26). Allergic reactions were frequent, occurring in 82% of children undergoing CM-OIT, although they were mostly mild and self-limiting. Nevertheless, the occurrence of anaphylaxis requiring the use of adrenaline was significantly more frequent (13%) in children undergoing CM-OIT compared to the avoidance diet or placebo (OR: 7.69, 95%CI: 2.16-27.33).

Protocols in which milk is administered orally are more effective than those using the sublingual route, but, on the other hand, are associated with a higher risk of adverse reactions (35,45). In a controlled study comparing the efficacy of sublingual immunotherapy with oral immunotherapy, including a total of 30 children (45), the authors demonstrated that oral immunotherapy after sublingual administration was more effective for desensitisation (60-80%, compared to only 10% in sublingual monotherapy); however, the frequency of systemic allergic reactions was higher with the oral immunotherapy.

Another approach that has been proposed to reduce the frequency of systemic allergic reactions in OIT is the fixed low-dose protocols (46), namely with a daily dose of 3mL of CM (46), compared to the usual target dose. However, the interest in these protocols will be only in terms of reducing the risk associated with accidental ingestion, as they do not allow for a CMP-free diet without restrictions, which is the primary goal of milk immunotherapy.

There is scarce information on long-term results, particularly regarding efficacy in terms of acquiring sustained unresponsiveness (3,24,35-37), and safety regarding the occurrence of severe allergic reactions and the use of adrenaline during the maintenance phase, and the possibility of eosinophilic esophagitis as an undesirable adverse effect after CM-OIT. In the review of studies, the frequency of anaphylaxis during maintenance phase ranges from 0 to 43% (24,25,33,47) and the frequency of eosinophilic esophagitis from 3 to 11% (24,27,44). It is expected that studies with longer follow-up times will be associated with higher frequencies of the disease (6,24). It is therefore expected that in the present study, with a follow-up of up to 15 years, the highest frequency of eosinophilic esophagitis was found (12%). The four patients who developed eosinophilic esophagitis as a side effect during the CM-OIT protocol, all underwent upper gastrointestinal endoscopy with esophageal biopsies and eosinophil counts prior to CM-OIT. The occurrence of eosinophilic esophagitis as a side effect in some patients undergoing CM-OIT (27,48), emphasises the importance of alerting patients to the possible onset of symptoms of esophageal dysfunction suggestive of eosinophilic esophagitis (49).

The meta-analysis published by WAO in 2022 (3), which included 13 randomized trials and 109 observational studies addressing cow's milk OIT, demonstrated a significant benefit in terms of efficacy in desensitisation to CMA (OR: 12.3; IC95%: 5.9-26), but reducing it to only 36% in terms of acquiring sustained unresponsiveness. CM-OIT increased the risk of anaphylaxis (OR: 60; IC95%: 15-244) and the frequency of adrenaline use (OR: 35.2; IC95%: 9-136.5) and was associated with an average frequency of 6.9% of eosinophilic esophagitis development.

Manuscript accepted for publication

During CM-OIT several cofactors can increase intestinal permeability, which is related to the occurrence of unexpected and potentially severe allergic reactions to previously well-tolerated doses (6,10,19,28). These reactions, which can occur at any stage of treatment, have been reported more frequently during the build-up phase and at the beginning of the maintenance phase, as well as when there was an increase in the daily dose of milk (after dose adjustment). For this reason, patients and their caregivers should be informed about the cofactors, and the important recommendations that must be followed to prevent allergic reactions during CM-OIT, which are listed in **Table V** (2,6,10,18,19,28,30). In our experience, adequate communication between the allergist and the patient and their caregivers, ensuring direct access to the medical team at any time, as well as information about risk factors and dose adjustment, when necessary, minimises the occurrence of allergic reactions (19).

The main cofactor in CM-OIT identified in this study, which is also the most described, is physical exercise (10,26,40). Physical exertion may be related to the occurrence of urticaria or even exercise-induced anaphylaxis dependent on CMP intake; for this reason, it should always be recommended to all patients a minimum interval of two hours between milk intake and vigorous physical exercise. Some guidelines recommend longer periods, namely up to three hours (10,30) and even up to four hours (2). However, in the authors' experience, a two-hour interval is sufficient, and prolonging the avoidance of physical exercise in children is not feasible in real-life.

In this study, all cases of CM exercise-induced anaphylaxis occurred when vigorous exercise took place within the 2-hour interval after milk intake (due to failure to comply with the interval or unforeseen circumstances). It should be noted that, in the authors' opinion, this recommendation should be followed until SPT and specific IgE becomes negative, as two adolescents had exercise-induced anaphylaxis at 6 and at 8 years of maintenance phase. Both these patients still had positive SPT and specific IgE to CM and casein.

Other cofactors we have found in CM-OIT have also been described by other authors, including fasting, acute infections (particularly respiratory or gastrointestinal viral infections), emotional stress, menstruation, and pollinic season (2,10,18,28,30). The family should carefully choose the preferred time for taking the daily dose of CM, which must be supervised by caregivers, ensuring that it is never given on an empty stomach and avoiding physical exercise in the following two hours. The possibility of unexpected allergic reactions, particularly associated with cofactors, justifies patients continuing to carry an AAI, even after completing the build-up phase. It is important to update the emergency plan, according to the child's weight, and to review how to use the AAI (8,12,31).

Oral immunotherapy with milk requires a long-term follow-up during the maintenance phase, with the need to ensure daily intake of the prescribed dose of CM (26,28,29,40). Most patients need to maintain daily exposure to CMP to ensure desensitisation. It should be noted that sustained unresponsiveness may not be achieved, and there is a state of desensitisation dependent on regular milk intake (28,29), so the patient and their caregivers must be continuously motivated to adhere to treatment. In the maintenance phase, the option of switching to milk derivatives (such as yoghurt) or other commercial food products with equivalent amounts of CMP (about 6.4 to 7 g per day), which may be more palatable to the patient, has proven to be an effective way of ensuring adherence to treatment (6,29).

Regarding dairy products, it is important to be aware of the problem with cheese, not only because of its high protein concentration but also because it may contain milk proteins from other mammalian species (particularly goat and sheep) to which the patient undergoing CM-OIT may react (6,10). When evaluating these patients, it is important to note that a high degree of cross-reactivity between milk caseins from different mammalian species is well documented in CMA, so the recommendation for CMA patients should be strict avoidance of CMP as well as goat and sheep milk (50).

As we did in this study, at the end of OIT, all patients should be tested for goat and sheep milk before allowing these to be introduced into their diet, except in cases where there is prior tolerance to these proteins, which is unusual (6,10). In the present study, during the maintenance phase, 15% of patients experienced anaphylaxis related to the accidental ingestion of goat or sheep milk

Manuscript accepted for publication

proteins, mainly in the form of cheese. And even with this protocol, which proved highly effective for CM, after completing the OIT, 49% of patients still showed allergic sensitisation to goat and sheep milk.

In this study, asthma was not identified as a risk factor for the occurrence of anaphylaxis during the maintenance phase of OIT. Although, asthma is a known risk factor for the severity and persistence of IgE-mediated CMA (6,9). And, it has been identified as a risk factor in CM-OIT, causing more severe and persistent allergic reactions during treatment (19,26,28,30).

In this prospective study lasting up to 15 years, it was possible to prove through *in vivo* and *in vitro* tests the existence of successful desensitisation, by assessing sensitisation to CM and to CMP before CM-OIT and after follow-up. There was a progressive reduction in skin reactivity (wheal diameter size in the SPT) in SPT to CM and to casein, and in specific IgE values to CM and to casein, with statistical significance. In most patients (61%) SPT became negative during the follow-up. Similarly, in a 4-year follow-up study of 21 children undergoing CM-OIT (39), the authors reported a decrease in serum specific IgE values for CM, and in six patients the SPT became negative. These markers were proposed by the authors as indicators of a good prognosis (39).

Other studies of CM-OIT have also demonstrated a progressive decrease in skin reactivity in SPT and in serum-specific IgE values for CM and casein, compared to baseline levels (26,28,29,32,40,47). Similarly, other prospective studies have also shown that SPT became negative with oral milk immunotherapy (28,32). This was accompanied by a gradual increase in serum-specific IgG4 values for CM and casein (21,28,29,33,40). In the present study, in twelve patients it was possible to measure specific IgG4 levels to CM and to casein before OIT, and when comparing baseline levels with the levels after OIT there was a significant increase. Therefore, negativization of SPT and reduction of specific IgE, as well as increase of specific IgG4, are considered by the authors as prognostic indicators of CM tolerance.

As limitations of the study, we highlight the fact that it is a non-controlled study, and that the existence of sustained unresponsiveness to CM was not evaluated. The absence of a control group means that we cannot guarantee with absolute certainty that some of our findings are in fact related to the efficacy of CM-OIT and do not depend on a spontaneous resolution of milk allergy.

The achievement of sustained unresponsiveness to CM was not evaluated in this study, as occurs in most studies. The main purpose of this study was the achievement of complete desensitisation to CM. In studies evaluating remission, this is usually assessed by performing an oral challenge after 2 to 4 weeks of interruption of contact with milk (3,29). Some patients can achieve sustained unresponsiveness, despite periods of withdrawal, while others need to fulfil regular milk consumption to maintain it. Some studies have reported loss of tolerance when ingestion is discontinued even on short periods. However, the minimal frequency of exposure to keep protection has not yet been established. It is not clear whether remission can be achieved in all allergic patients, provided OIT is maintained long enough (6,10). Meaning the maintenance period needed to achieve sustained unresponsiveness is not known (6,10).

Follow-up of patients undergoing milk immunotherapy should be maintained until loss of allergenic sensitisation documented by negative SPT and specific IgE, or until a state of sustained unresponsiveness is achieved, confirmed by a period of milk avoidance of about four weeks, followed by a negative oral challenge (28). Performance of this oral challenge is not usually done in real-life during follow-up, due to the risk of jeopardising the state of desensitisation acquired during OIT (6).

It is important to emphasise that CM-OIT should be used judiciously, always in specialised hospital centres, with the necessary differentiation and ongoing support from a medical team of experienced allergists, ensuring maximum safety and efficacy of the OIT (6,10,12,18,30). Other necessary conditions include the availability, motivation and understanding of objectives by the patient and their caregivers, as well as informed knowledge of the risks and benefits of OIT and the need for prolonged maintenance therapy with CMP for several years (6,10,19,29).

CONCLUSIONS

Manuscript accepted for publication

This study confirms the high efficacy of CM-OIT and the persistence of its protective effect over time, provided that regular intake of CMP is maintained. Adherence to the daily maintenance dose of 200mL of CM or equivalent is essential to ensure desensitisation.
Allergic reactions are frequent during the maintenance phase, and the possibility of anaphylaxis in an outpatient setting is a reality, but continuous contact with the medical team has made it possible to minimise these situations through rapid and effective management of these episodes. It is important that patients and their caregivers are aware of the main cofactors and follow the relevant recommendations.
A shared decision-making process for CM-OIT should be considered on a case-by-case basis, engaging the family (and the patient, where applicable) and the allergist. Milk immunotherapy should be conducted by experienced allergists, under appropriate hospital conditions, and reserved for specialised centers. The cornerstone of successful CM-OIT is the optimisation of the established doctor-patient/family relationship, with a medical team permanently available and able to respond quickly, and well-informed and motivated patients and caregivers. Continuous access to the medical team, as well as awareness of potential cofactors and dose adjustments whenever necessary, minimise the occurrence and severity of allergic reactions during OIT and increase adherence to treatment.

Funding
The authors declare that no funding was received.
Contributions
AG: Conceptualization, Data curation, Formal Analysis, Methodology, Writing - original draft, Writing - review & editing; SP: Conceptualization, Data curation, Methodology, Writing - review & editing; CMM, IM and LMB: Writing - review & editing.
Conflicts of interest: The authors declare that they have no conflicts of interest.

Acknowledgments
The authors are grateful to Professor Catarina Martins (from NOVA Medical School/Comprehensive Health Research Center) for statistical analysis. The authors are also grateful to the children who participated in the study, and to their parents, without whose cooperation the study would not have been possible.

REFERENCES
1. Lyons SA, Clausen M, Knulst AC, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, *et al*. Prevalence of Food Sensitization and Food Allergy in Children Across Europe. J Allergy Clin Immunol Pract. 2020;8(8):2736-46.e9. doi: 10.1016/j.jaip.2020.04.020.
2. Brozek JL, Firmino RT, Bognanni A, Arasi S, Ansotegui I, Assa'ad AH, *et al*. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guideline update - XIV - Recommendations on CMA immunotherapy. World Allergy Organ J. 2022;15(4):100646. doi: 10.1016/j.waojou.2022.100646.
3. Bognanni A, Chu DK, Firmino RT, Arasi S, Waffenschmidt S, Agarwal A, *et al*; WAO DRACMA Guideline Group. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) guideline update - XIII - Oral immunotherapy for CMA - Systematic review. World Allergy Organ J. 2022;15(9):100682. doi: 10.1016/j.waojou.2022.100682.
4. Spolidoro GCI, Ali MM, Amera YT, Nyassi S, Lisik D, Ioannidou A, *et al*. Prevalence estimates of eight big food allergies in Europe: Updated systematic review and meta-analysis. Allergy. 2023;78(9):2361-417. doi: 10.1111/all.15801.
5. Gaspar-Marques J, Carreiro-Martins P, Papoila AL, Caires I, Pedro C, Araújo-Martins J, *et al*. Food allergy and anaphylaxis in infants and preschool-age children. Clin Pediatr (Phila). 2014;53(7):652-7. doi: 10.1177/0009922814527502.

Manuscript accepted for publication

6. Gaspar A, Costa C, Piedade S, Prates S, Carrapatoso I, Reis-Ferreira A. Chapter IV: Oral immunotherapy with milk. Guidelines of oral immunotherapy for food allergy. Rev Port Imunoalergologia. 2025;33(1):9-26. doi: 10.32932/rpia.2025.03.153.
7. Gaspar A, Santos N, Faria E, Pereira AM, Gomes E, Câmara R, *et al*. Anaphylaxis in children and adolescents: The Portuguese Anaphylaxis Registry. Pediatr Allergy Immunol. 2021;32(6):1278-86. doi: 10.1111/pai.13511.
8. Gaspar A, Lozoya C, Carrapatoso I, Prates S, Piedade S, Reis-Ferreira A. Chapter I: From epidemiology to management of food allergy. Guidelines of oral immunotherapy for food allergy. Rev Port Imunoalergologia. 2024;32(4):187-94. doi: 10.32932/rpia.2024.12.148.
9. Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. J Allergy Clin Immunol. 2007;120(5):1172-7. doi: 10.1016/j.jaci.2007.08.023.
10. Martorell A, Alonso E, Echeverría L, Escudero C, García-Rodríguez R, Blasco C, *et al*; Expert panel selected from members of the Spanish Society of Pediatric Allergology, Asthma and Clinical Immunology (SEICAP) and the Spanish Society of Allergology and Clinical Immunology (SEAIC). Oral Immunotherapy for Food Allergy: A Spanish Guideline. Immunotherapy Egg and Milk Spanish Guide (ITEMS Guide). Part I: Cow Milk and Egg Oral Immunotherapy: Introduction, Methodology, Rationale, Current State, Indications, Contraindications, and Oral Immunotherapy Build-up Phase. J Investig Allergol Clin Immunol. 2017;27(4):225-37. doi: 10.18176/jiaci.0177.
11. Golding MA, Batac ALR, Gunnarsson NV, Ahlstedt S, Middelveld R, Protudjer JLP. The burden of food allergy on children and teens: A systematic review. Pediatr Allergy Immunol. 2022;33(3):e13743. doi:10.1111/pai.13743.
12. Santos AF, Riggioni C, Agache I, Akdis CA, Akdis M, Alvarez-Perea A, *et al*. EAACI guidelines on the management of IgE-mediated food allergy. Allergy. 2025;80(1):14-36. doi: 10.1111/all.16345.
13. Santos A, Dias A, Pinheiro JA. Predictive factors for the persistence of cow's milk allergy. Pediatr Allergy Immunol. 2010;21(8):1127-34. doi: 10.1111/j.1399-3038.2010.01040.x.
14. Santos AF, Riggioni C, Agache I, Akdis CA, Akdis M, Alvarez-Perea A, et al. EAACI guidelines on the diagnosis of IgE-mediated food allergy. Allergy. 2023;78(12):3057-3076. doi: 10.1111/all.15902.
15. Baseggio Conrado A, Ierodiakonou D, Gowland MH, Boyle RJ, Turner PJ. Food anaphylaxis in the United Kingdom: analysis of national data, 1998-2018. BMJ. 2021;372:n251. doi: 10.1136/bmj.n251.
16. Baseggio Conrado A, Patel N, Turner PJ. Global patterns in anaphylaxis due to specific foods: a systematic review. J Allergy Clin Immunol. 2021;148(6):1515-25.e3. doi: 10.1016/j.jaci.2021.03.048.
17. Fiocchi A, Bognanni A, Brożek J, Ebisawa M, Schünemann H; WAO DRACMA guideline group. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guidelines update - I - Plan and definitions. World Allergy Organ J. 2022;15(1):100609. doi: 10.1016/j.waojou.2021.100609.
18. Muraro A, de Silva D, Halken S, Worm M, Khaleva E, Arasi S, *et al*; GA^{2}LEN Food Allergy Guideline Group. Managing food allergy: GA^{2}LEN guideline 2022. World Allergy Organ J. 2022;15(9):100687. doi: 10.1016/j.waojou.2022.100687.
19. Gaspar A, Prates S, Piedade S, Carrapatoso I, Reis-Ferreira A. Chapter II: Basic principles of oral immunotherapy with food. Guidelines of oral immunotherapy for food allergy. Rev Port Imunoalergologia. 2024;32(4):195-208. doi: 10.32932/rpia.2024.12.149.
20. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro P. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. Allergy 2004;59(9):980-7. doi: 10.1111/j.1398-9995.2004.00542.x.
21. Pajno GB, Caminiti L, Ruggeri P, De Luca R, Vita D, La Rosa M, *et al*. Oral immunotherapy for cow's milk allergy with a weekly up-dosing regimen: a randomized single-blind controlled study. Ann Allergy Asthma Immunol. 2010;105(5):376-81. doi: 10.1016/j.anai.2010.03.015.

Manuscript accepted for publication

22. Martorell A, De la Hoz B, Ibáñez MD, Bone J, Terrados MS, Michavila A, *et al*. Oral desensitization as a useful treatment in 2-year-old children with cow's milk allergy. Clin Exp Allergy. 2011;41(9):1297-304. doi: 10.1111/j.1365-2222.2011.03749.x.
23. Morais-Almeida M, Piedade S, Couto M, Sampaio G, Santa-Marta C, Gaspar A. Innovation in specific oral tolerance induction in children with anaphylaxis to cow's milk proteins. Rev Port Imunoalergologia. 2011;19(3):161-9.
24. Salmivesi S, Korppi M, Mäkelä MJ, Paassilta M. Milk oral immunotherapy is effective in school-aged children. Acta Paediatr. 2012;102(2):172-6. doi: 10.1111/j.1651-2227.2012.02815.x.
25. Lee JH, Kim WS, Kim H, Hahn YS. Increased cow's milk protein-specific IgG4 levels after oral desensitization in 7- to 12-month-old infants. Ann Allergy Asthma Immunol. 2013;111(6):523-8. doi: 10.1016/j.anai.2013.09.001.
26. Mota I, Piedade S, Gaspar A, Benito-Garcia F, Sampaio G, Borrego LM, *et al*. Cow's milk oral immunotherapy in real life: 8-year long-term follow-up study. Asia Pac Allergy. 2018;8(3):e28. doi: 10.5415/apallergy.2018.8.e28.
27. Schryver S, Mazer B, Clarke AE, Pierre Y, Lejtenyi D, Langlois A, et al. Adverse Events in Oral Immunotherapy for the Desensitization of Cow's Milk Allergy in Children: A Randomized Controlled Trial. J Allergy Clin Immunol Pract. 2019;7(6):1912-9. doi: 10.1016/j.jaip.2019.02.007.
28. Martorell A, Alonso E, Echeverría L, Escudero C, García-Rodríguez R, Blasco C, *et al*; Expert panel selected from members of the Spanish Societies of Pediatric Allergology, Asthma and Clinical Immunology (SEICAP) and Allergology and Clinical Immunology (SEAIC). Oral immunotherapy for food allergy: A Spanish guideline. Egg and milk immunotherapy Spanish guide (ITEMS GUIDE). Part 2: Maintenance phase of cow milk (CM) and egg oral immunotherapy (OIT), special treatment dosing schedules. Models of dosing schedules of OIT with CM and EGG. Allergol Immunopathol (Madr). 2017;45(5):508-18. doi: 10.1016/j.aller.2017.05.002.
29. Laubach S, Kim EH, Greenhawt M, Bailey S, Anagnostou A. A Review of Shared Decision-Making, Published Protocols, and Post-desensitization Strategies in Oral Immunotherapy (OIT). Curr Allergy Asthma Rep. 2024;24(4):173-97. doi: 10.1007/s11882-024-01132-2.
30. Pajno GB, Fernandez-Rivas M, Arasi S, Roberts G, Akdis CA, Alvaro-Lozano M, *et al*; EAACI Allergen Immunotherapy Guidelines Group. EAACI Guidelines on allergen immunotherapy: IgE-mediated food allergy. Allergy. 2018;73(4):799-815. doi: 10.1111/all.13319.
31. Muraro A, Worm M, Alviani C, Cardona V, DunnGalvin A, Garvey LH, *et al*. EAACI guidelines: Anaphylaxis (2021 update). Allergy. 2022;77(2):357-77. doi: 10.1111/all.15032.
32. Martorell-Aragonés A, Félix Toledo R, Cerdá Mir JC, Martorell Calatayud A. Oral rush desensitization to cow milk. Following of desensitized patients during three years. Allergol Immunopathol (Madr). 2007;35(5):174-6. doi: 10.1157/13110311.
33. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, *et al*. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. J Allergy Clin Immunol. 2008;122(6):1154-60. doi: 10.1016/j.jaci.2008.09.030.
34. Dantzer J, Dunlop J, Psoter KJ, Keet C, Wood R. Efficacy and safety of baked milk oral immunotherapy in children with severe milk allergy: A randomized, double-blind, placebo-controlled phase 2 trial. J Allergy Clin Immunol. 2022;149(4):1383-91.e17. doi: 10.1016/j.jaci.2021.10.023.
35. Nurmatov U, Dhami S, Arasi S, Pajno GB, Fernandez-Rivas M, Muraro A, *et al*. Allergen immunotherapy for IgE-mediated food allergy: a systematic review and meta-analysis. Allergy. 2017;72(8):1133-47. doi: 10.1111/all.13124.
36. Tang L, Yu Y, Pu X, Chen J. Oral immunotherapy for Immunoglobulin E-mediated cow's milk allergy in children: A systematic review and meta analysis. Immun Inflamm Dis. 2022;10(10):e704. doi: 10.1002/iid3.704.

Manuscript accepted for publication

37. Rodríguez Del Río P, Álvaro-Lozano M, Arasi S, Bazire R, Escudero C, Patel N, *et al*. Evaluation of clinical outcomes of efficacy in food allergen immunotherapy trials, COFAITH EAACI task force. Allergy. 2024;79(4):793-822. doi: 10.1111/all.16027.
38. Riggioni C, Oton T, Carmona L, Du Toit G, Skypala I, Santos AF. Immunotherapy and biologics in the management of IgE-mediated food allergy: Systematic review and meta-analyses of efficacy and safety. Allergy 2024;79(8):2097-127. doi: 10.1111/all.16129.
39. Meglio P, Giampietro PG, Gianni S, Galli E. Oral desensitization in children with immunoglobulin E-mediated cow's milk allergy - follow-up at 4 yr and 8 months. Pediatr Allergy Immunol. 2008;19(5):412-9. doi: 10.1111/j.1399-3038.2007.00670.x.
40. Alves-Correia M, Gaspar A, Borrego LM, Azevedo J, Martins C, Morais-Almeida M. Successful oral desensitization in children with cow's milk anaphylaxis: Clinical and laboratory evaluation up to nine-years follow-up. Allergol Immunopathol (Madr). 2019;47(2):133-40. doi: 10.1016/j.aller.2018.07.007.
41. Nowak-Wegrzyn A, Fiocchi A. Rare, medium, or well done? The effect of heating and food matrix on food protein allergenicity. Curr Opin Allergy Clin Immunol. 2009;9(3):234-7. doi: 10.1097/ACI.0b013e32832b88e7.
42. Gruzelle V, Juchet A, Martin-Blondel A, Michelet M, Chabbert-Broue A, Didier A. Benefits of baked milk oral immunotherapy in French children with cow's milk allergy. Pediatr Allergy Immunol. 2020;31(4):364-70. doi: 10.1111/pai.13216.
43. Anagnostou A, Mack DP, Johannes S, Shaker M, Abrams EM, DeSanto K, *et al*. The Safety and Efficacy of Baked Egg and Milk Dietary Advancement Therapy: A Systematic Review and Meta-Analysis. J Allergy Clin Immunol Pract. 2024;12(9):2468-80. doi: 10.1016/j.jaip.2024.06.016.
44. Nagakura KI, Sato S, Miura Y, Nishino M, Takahashi K, Asaumi T, *et al*. A randomized trial of oral immunotherapy for pediatric cow's milk-induced anaphylaxis: Heated vs unheated milk. Pediatr Allergy Immunol. 2021;32(1):161-9. doi: 10.1111/pai.13352.
45. Keet CA, Frischmeyer-Guerrerio PA, Thyagarajan A, Schroeder JT, Hamilton RG, Boden S, *et al*. The safety and efficacy of sublingual and oral immunotherapy for milk allergy. J Allergy Clin Immunol. 2012;129(2):448-55.e1-5. doi: 10.1016/j.jaci.2011.10.023.
46. Miura Y, Nagakura KI, Nishino M, Takei M, Takahashi K, Asaumi T, *et al*. Long-term follow-up of fixed low-dose oral immunotherapy for children with severe cow's milk allergy. Pediatr Allergy Immunol. 2021;32(4):734-41. doi: 10.1111/pai.13442.
47. Maeda M, Imai T, Ishikawa R, Nakamura T, Kamiya T, Kimura A, *et al*. Effect of oral immunotherapy in children with milk allergy: The ORIMA study. Allergol Int. 2021;70(2):223-8. doi: 10.1016/j.alit.2020.09.011.
48. Pitsios C, Rossi CM, Terreehorst I, Heffler E, Votto M, Konstantinou GN, *et al*. Eosinophilic esophagitis as a side-effect of allergen immunotherapy: protocol for a systematic review and meta-analysis. Eur Ann Allergy Clin Immunol. 2024;56(1):4-8. doi: 10.23822/EurAnnACI.1764-1489.311.
49. Amil-Dias J, Oliva S, Papadopoulou A, Thomson M, Gutiérrez-Junquera C, Kalach N, *et al*. Diagnosis and management of eosinophilic esophagitis in children: An update from the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). J Pediatr Gastroenterol Nutr. 2024;79(2):394-437. doi: 10.1002/jpn3.12188.
50. Restani P, Gaiaschi A, Plebani A, Beretta B, Cavagni G, Fiocchi A, et al. Cross-reactivity between milk proteins from different animal species. Clin Exp Allergy. 1999;29(7):997-1004. doi: 10.1046/j.1365-2222.1999.00563.x.

Manuscript accepted for publication

Table I - Eligibility criteria for cow's milk oral immunotherapy (2,6,10,18,30)

Indications: **I. Persistent IgE-mediated CMA**: - Confirmed: recent history of systemic allergic reaction after ingestion (last year) or positive oral challenge with cow's milk; - IgE-mediated reaction: evidence of allergic sensitisation (documented by positive skin prick tests and serum specific IgE). **II. Severe CMA**: - Previous severe reactions to cow's milk; - Impaired in the quality of life of the patient and family.
Necessary conditions: - Informed consent: Parents informed about all aspects of the protocol in the short and long term, namely the risk of allergic reactions and the need for prolonged maintenance of daily milk intake; - Good family adherence to treatment and follow-up; - Stability: in the place of residence and family situation; - Caregivers' ability to adequately manage allergic reactions; - Medical team experienced in this type of procedure; - Appropriate physical space: day hospital facilities.
Absolute contraindications: - Non IgE-mediated CMA; - Previous diagnosis of eosinophilic esophagitis or other eosinophilic gastrointestinal disorders; - Uncontrolled asthma (disease must be controlled before OIT is started); - Family unable to adhere to the protocol or unable to identify and treat allergic reactions appropriately; - Neoplasia or systemic immunosuppression therapy; - Active systemic autoimmune disorders; - Initiation during pregnancy.
Relative contraindications: - Uncontrolled active atopic dermatitis / chronic urticaria; - Systemic mastocytosis; - Inflammatory bowel disease; - Systemic autoimmune disorders in remission or organ specific (i.e. thyroiditis); - Therapy with beta-blockers (non-cardioselective) or ACE inhibitors.

Manuscript accepted for publication

Table II - Dosing schedule of the build-up phase of our cow's milk sublingual-oral immunotherapy protocol (23)

Session 1 (Day 1)	**Session 2** (Day 14 to 28)	**Session 3** (Day 28 to 56)	**Session 4** (Day 42 to 70)	**Session 5** (Day 56 to 84)
1 drop * 2 drops * 3 drops * 4 drops * 0.1 mL 0.2 mL 0.5 mL 0.5 mL 1 mL	1 mL 5 mL 5 mL 10 mL 10 mL	10 mL 10 mL 20 mL 20 mL 50 mL	50 mL 50 mL (interval 2h) 100 mL	100 mL 100 mL (interval 2h) 200 mL
Interval between doses 20-30 minutes (***sublingual doses**)			Interval 20-120 minutes	
Maintenance dose (at home)				
0.5 to 1 mL twice-daily	5 to 10 mL twice-daily	20 to 50 mL twice-daily	100 mL twice-daily	**200 mL daily** (…progressive free diet)
Mild or isolated symptoms: continue protocol after symptoms subside - possible prescription of antihistamines and adjustment/repetition of doses. Anaphylaxis or persistent symptoms: treat accordingly, restart with previous dose and increase according to response and tolerance.				

Table III - Characterization of the 33 patients who underwent cow's milk oral immunotherapy and were on follow-up study

Descriptive data	(n = 33)
Sex (male)	19 (58%)
History of CM anaphylaxis (≥ 3 episodes of anaphylaxis - 9)	22 (67%)
Asthma	23 (70%)
Allergic rhinitis	32 (97%)
Atopic dermatitis	12 (36%)
Other food allergy (egg - 8, tree nuts - 5, kiwi - 4, peanut - 3, fish - 2 and shellfish - 2)	10 (30%)
Total IgE (median, range) (kU/L)	200 (15.3-1258)
Atopy	27 (82%)
CM-OIT (build-up phase) Mean age at CM-OIT initiation (±SD) Mean period until target dose (±SD)	 7.0 (±2.8) years (range: 2-16 years) 5.8 (±2.5) months (range: 2-12 months)
Anaphylaxis during build-up phase OIT	7 (21%)
CM-OIT (maintenance phase) Mean age at beginning (±SD)	 7.5 (±2.9) years

Manuscript accepted for publication

Mean age at last clinical evaluation (±SD) Mean time of follow-up (±SD)	(range: 3-16 years) 15.8 (±5.5) years (range: 8-27 years) 8.0 (±4.4) years (range: 1-15 years)
Sensitization to goat's milk and sheep's milk after OIT	16 (49%)
Allergic reactions during maintenance phase OIT (≥ 3 episodes - 19)	26 (79%)
Anaphylaxis during maintenance phase OIT	11 (33%)
Use of adrenaline autoinjector during OIT (including build-up phase and maintenance phase)	6 (18%)

Atopy: defined as the existence of at least one positive skin prick test or specific IgE to common aeroallergens (dust mites, pollens, molds, cat or dog).
CM: cow's milk; OIT: oral immunotherapy; SD: standard deviation

Table IV - Skin prick tests and specific IgE levels to cow´s milk and casein before and after cow's milk oral immunotherapy: in all patients, in patients with anaphylaxis during maintenance phase and in patients without anaphylaxis during maintenance phase. When available, specific IgG4 levels to cow´s milk and casein before and after immunotherapy

	Before OIT	**After OIT**	**p**
Cow's milk SPT (wheal, mm)			
All patients, mean (±SD) median (range)	8.92 (±3.85) 8.50 (3.0-19.50)	0.0 (0.0-6.50) *	<0.0001
Patients with anaphylaxis after OIT	7.36 (±3.19) 6.0 (5.0-15.0)		n.s.
Patients without anaphylaxis after OIT	9.68 (±3.99) 10.0 (3.0-19.50)		
Casein SPT (wheal, mm)			
All patients, mean (±SD) median (range)	10.35 (±4.26) 10.0 (4.0-21.50)	0.0 (0.0-7.50) *	<0.0001
Patients with anaphylaxis after OIT	8.95 (±2.79) 10.0 (5.0-14.0)		n.s.
Patients without anaphylaxis after OIT	11.0 (±4.49) 10.0 (4.0-21.50)		
Cow's milk IgE levels (kU/L)			
All patients, mean (±SD) median (range)	23.02 (±19.84) 17.75 (0.51-78.90)	2.20 (0.0-22.20) #	<0.0001
Patients with anaphylaxis after OIT	33.36 (±20.75) 29.75 (12.3-78.90)		n.s.
Patients without anaphylaxis after OIT	19.09 (±17.54) 14.0 (0.51-64.20)		
Casein IgE levels (kU/L)			
All patients, mean (±SD) median (range)	17.34 (±18.15) 11.35 (0.40-75.80)	0.85 (0.0-23.70) #	<0.0001
Patients with anaphylaxis after OIT	24.15 (±19.59) 19.95 (2.90-75.80)		n.s.
Patients without anaphylaxis after OIT	14.88 (±16.67)		

Manuscript accepted for publication

	6.0 (0.40-51.40)		
Cow's milk IgG4 levels (mg/L) [&]			
All patients, mean (±SD) median (range)	8.55 (±4.42) 8.60 (0.46-17.50)	20.10 (±12.53) 30.0 (1.0-30.0)	0.0353
Casein IgG4 levels (mg/L) [&]			
All patients, mean (±SD) median (range)	2.71 (±3.72) 0.67 (0.07-9.50)	16.25 (±12.65) 11.45 (0.33-30.0)	<0.001

* 20 patients had negative SPT to cow's milk and casein levels after OIT.
[#] 12 patients had negative IgE levels to cow's milk and casein after OIT.
[&] IgG4 specific levels to cow's milk and casein were performed before OIT in 12 patients. IgG4 specific levels to cow's milk and casein were performed after OIT in 25 patients.
n.s.: not statistically significant; OIT: oral immunotherapy; SD: standard deviation; SPT: skin prick tests with allergen extracts

Table V - Recommendations during cow's milk oral immunotherapy, to prevent allergic reactions related with cofactors (2,6,10,18,19,28,30)

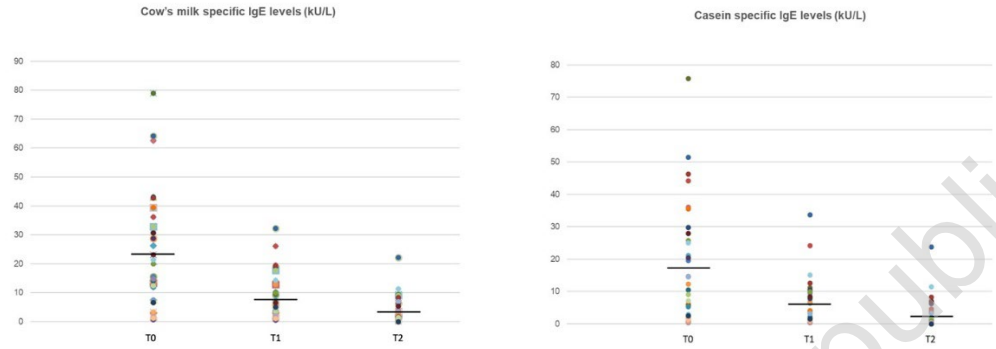
Recommendations during cow's milk oral immunotherapy (indispensable in the build-up phase and at the first years of the maintenance phase)
- Never in fasting, always administer the milk dose with food
- Avoid physical exercise in the 2 hours after the milk dose until SPT and specific IgE to CMP became negative *
- Avoid NSAID in the period of 2 to 3 hours around the milk dose
- Avoid hot bath in the 2 hours after taking the milk dose
- Avoid sleeping 1 to 2 hours after taking the milk dose
In the event of an intercurrent disease: **- Asthma exacerbation**: 50% reduction of the milk dose; **- Gastroenteritis**: 50% dose reduction, or if necessary, due to vomiting, suspension of milk during the acute phase of the disease for a maximum period of 3 days, with return in hospital environment with 50% reduction of the milk dose; **- Febrile upper airway infection**: return to the previous milk tolerated dose.
- Avoid sheep, goat and other mammalian milk proteins until SPT and specific IgE to these proteins became negative *

* based on the authors' experience

CMP: cow's milk proteins; NSAID: nonsteroidal anti-inflammatory drugs; SPT: skin prick tests

Manuscript accepted for publication

Figure 1 – (A) Evolution over time of serum levels of specific IgE for cow's milk, immediately before beginning OIT (T0), after the first year of follow-up (T1) and at the last assessment after OIT (T2), with the respective mean (T0: 23.02 kU/L; T1: 7.88 kU/L; T2: 2.20 kU/L); **(B)** Evolution over time of serum levels of specific IgE for casein, immediately before beginning OIT (T0), after the first year of follow-up (T1) and at the last assessment after OIT (T2), with the respective mean (T0: 17.34 kU/L; T1: 5.87 kU/L; T2: 0.85 kU/L)



Manuscript accepted for publication