PROGRAMA DE DOCTORADO EN INVESTIGACIÓN
EN CIENCIAS DE LA SALUD

TESIS DOCTORAL:

COMPARACIÓN RETROSPECTIVA DEL MANEJO DE LA ALERGIA A LAS PROTEÍNAS DE LECHE DE VACA MEDIADA POR IGE EN TRES COHORTES

RETROSPECTIVE COMPARISON OF IGE-MEDIATED COWS MILK PROTEIN ALLERGY MANAGEMENT IN THREE COHORTS

Presentada por

Juan Enrique Trujillo Wurttele

para optar al grado de

Doctor por la Universidad de Valladolid

Dirigida por:

Dr. Roberto Velasco Zúñiga

Año 2023

Agradecimientos

Deseo expresar mi más sincero agradecimiento a Roberto quien, un excelente investigador y director de tesis, me ha orientado y apoyado en todos los aspectos de la realización de esta tesis doctoral desde sus inicios.

Igualmente, a todos los profesionales de los diferentes centros de investigación que han colaborado en la recogida de datos y que han logrado que sea posible realizar este trabajo.

A University College Cork (UCC) y Cork University Hospital (CUH) centros de los cuales estoy orgulloso de pertenecer y que han conseguido que logre superarme y conseguir mi objetivo.

Gracias a Paola, que ha logrado apoyarme en los momentos más difíciles de este camino.

Por último, y con nombre propio de las personas que me han inspirado desde mis inicios en pediatría y alergia para lograr pensar en que uno puede lograr lo que se propone: José (Pepe), Frances, Carlos, Monserrat, Jonathan, Deirdre, Liam y Caoimhe.

Index

SUMMARY 11

1. INTRODUCTION 15

1.1 The burden of food allergies 17

1.2 Clinical manifestations of CMPA 18

1.2.1 Types of allergic reaction 18

1.3 Allergens 20

1.3.1 Major and relevant minor allergenic molecules 20

1.3.2 Heat stability of major allergens and clinical relevance 21

1.4 CMPA Resolution 21

1.5 Diagnosis of CMPA 23

1.6 Prevention of CMPA 25

1.7 Treatment of CMPA 26

1.7.1 Strict Avoidance of CM and derivatives 26

1.7.2 Role of baked milk and the use of food ladder/ home re-introduction milk strategy 27

1.7.3 Oral Immunotherapy 31

1.7.4 Early introduction of milk using an OIT strategy in young infants 36

1.7.5 General view of the evolution in the treatment of CMPA 39

1.8 The situation of allergy and differences in management in two European countries 41

1.8.1 Ireland 41

1.8.2 Spain 42

1.8.3 Difference in management 43

2. OBJECTIVES AND ENDPOINTS 45

2.1 Primary objective 47

2.2 Secondary objectives 47

3. MATERIALS AND METHODS **49**

3.1 Study design **51**

3.2 Definitions **52**

3.3 Management in each centre **53**

3.3.1 Milk Ladder 54

3.3.2 Strict Milk avoidance 56

3.3.3 Early gradual reintroduction of milk 57

3.4 Patient selection and data management **58**

3.4.1 Inclusion Criteria 58

3.4.2 Exclusion Criteria 58

3.5 Development of the research study **59**

3.6 Compliance/ Governance and Ethical issues **60**

3.7 Statistical methods **61**

4. RESULTS **63**

4.1 General Data **65**

4.2 Demographic and baseline characteristics **66**

4.3 Successful reintroduction and safety **68**

4.4 Relationship between the immunological parameters and the success of the treatment **71**

4.4.1 Skin Prick test 71

4.4.2 Specific IgE 72

4.5 Association with relevant medical history of allergy **73**

4.6 Association between other food allergies and success in the treatment **74**

4.7 Association between other allergies and success in the treatment **76**

4.8 Overall analysis **78**

5. DISCUSSION 79

5.1 A strong progression to an active treatment of CMPA 81

5.1.1 Milk avoidance 81

5.1.2 The use of Milk reintroduction Milk Ladder 82

5.1.3 Early gradual reintroduction of milk 82

5.2 Achieving our primary objective 83

5.3 Comparing the characteristics of each management 84

5.4 Safety of each protocol 85

5.4 Can we rely in the usual parameter to predict an outcome? 87

5.4.1 Immunological parameter 87

5.4.2 Association with other allergies 89

5.5 Limitations 90

6. CONCLUSIONS 91

7. REFERENCES 95

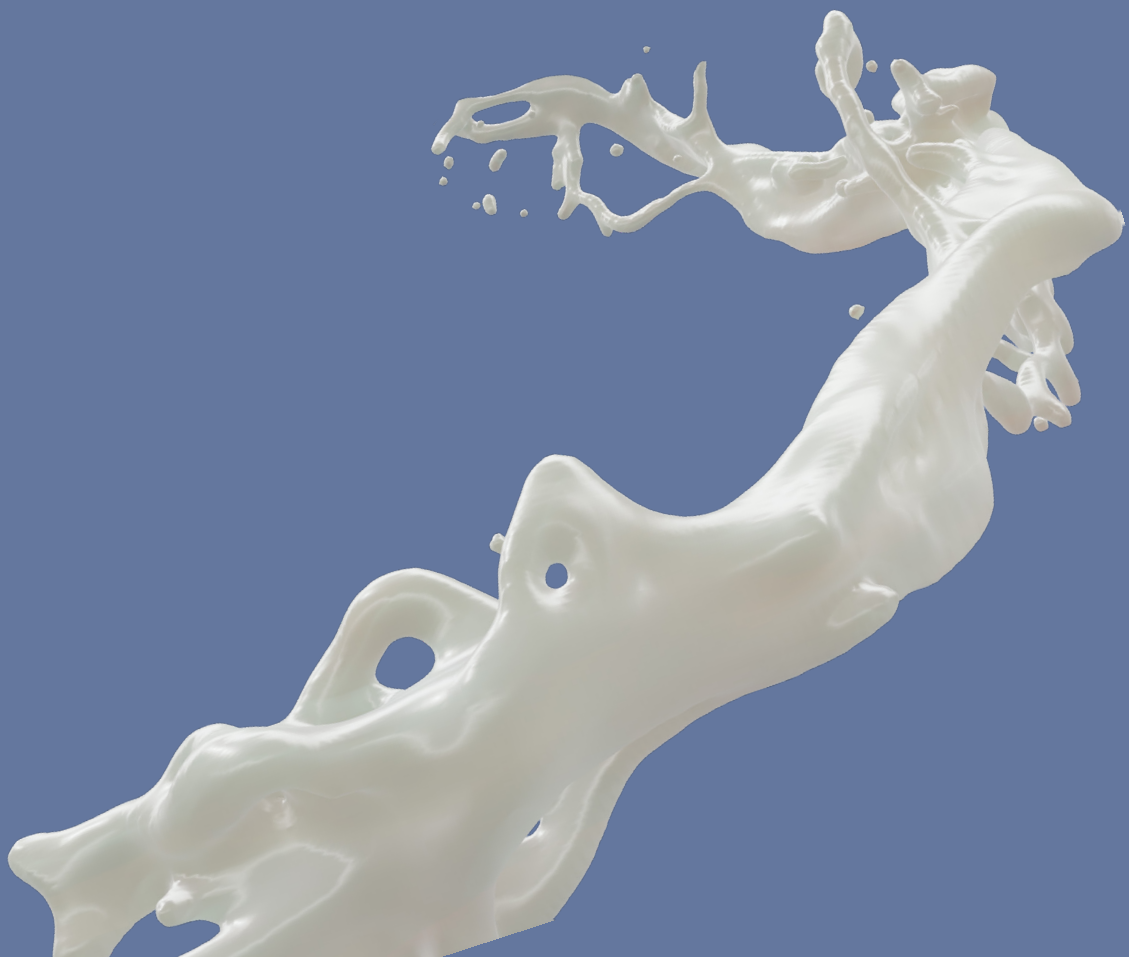
9. ATTACHMENTS 105

9.1 Glossary of Terms 107

9.2 Survey of each research centre 108

9.3 Ethics committee approval 110

SUMMARY

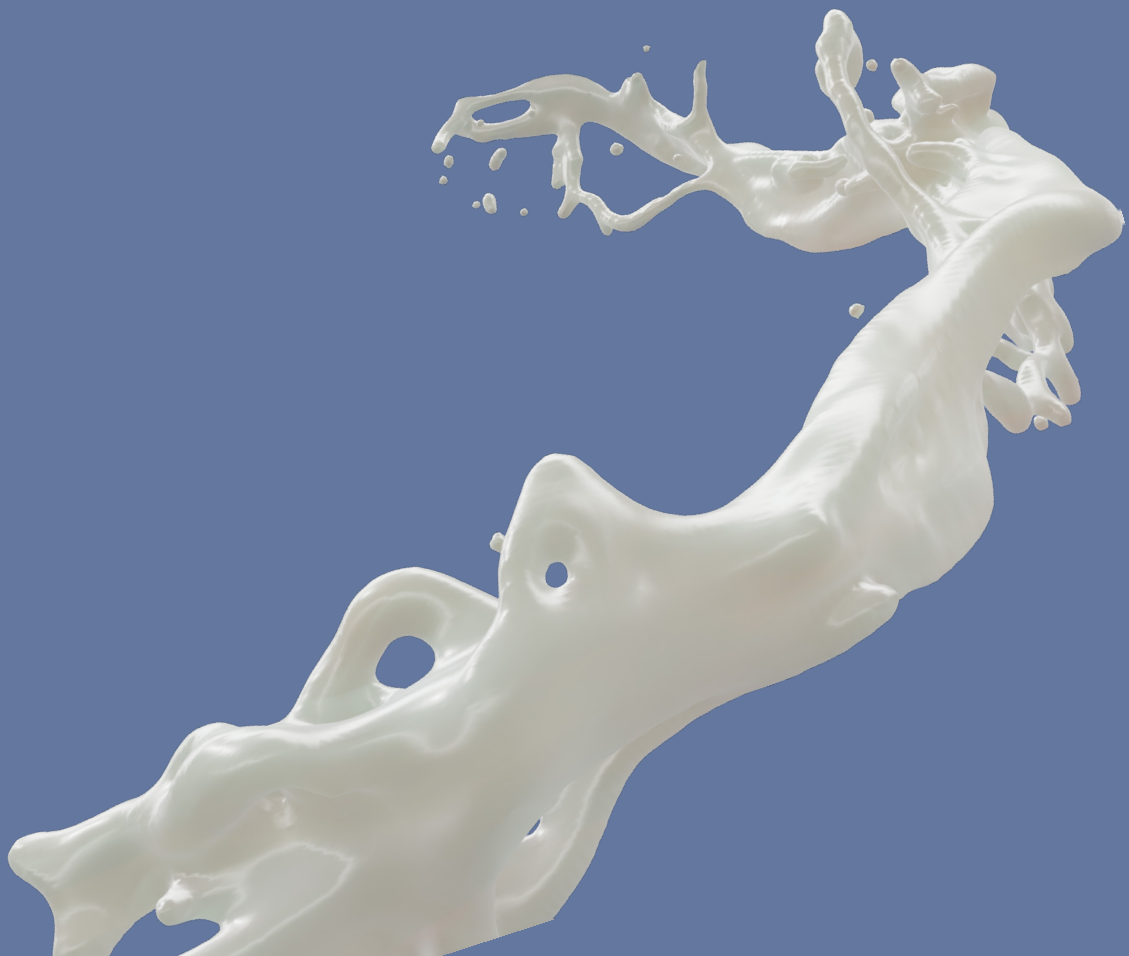


Summary

IgE mediated Cow's milk protein Allergy (CMPA) is one of the most common causes of food allergy among paediatric patients across the globe. The classical approach to management of this disease is the avoidance of milk products until they naturally outgrow the allergy and can introduce milk into their diet. In the last decade, other novel ways of treating CMPA have been arising in different allergy centres across Europe, including home introduction of milk using an escalated milk product plan "Milk Ladder" and "early gradual milk introduction" in hospital, with continued treatment as outpatient, giving a new, more active role to caregivers and health care workers.

The main objective of this project is to compare the rate of acquired full reintroduction of milk of three different IgE-mediated CMPA treatment strategies.

1. INTRODUCTION



1 Introduction

1.1 The burden of food allergies

Allergy is a major problem in our society. It constitutes a significant cause of morbidity worldwide and is a considerable burden on the health and medical systems of both developed and emerging economies. Allergic diseases include a broad spectrum of conditions that includes asthma, rhinosinusitis, atopic dermatitis and life-threatening food, drug, and stinging insect allergies. They affect at least 30% of the population and nearly 80% of families (1). All allergic diseases are characterized by a distinct pattern of inflammation that is largely driven via immunoglobulin E (IgE)-dependent mechanisms (2).

Food allergies (FA) are an emerging healthcare issue. There are extensive data that document the prevalence rate to be as high as approximately 10% (3). There are several differences in the prevalence of different food allergies. However, most countries reported an increase in food allergy prevalence (4). In the case of Ireland, it affects 4% of infants, with the dominant foods being cow's milk, egg and peanut with similar values being found in different countries in Europe (5).

Cow's milk protein allergy (CMPA) is one of the most common food allergies in infancy and childhood, affecting approximately 1% of Irish infants and between 0.3 and 7.5% of infants in Spain (6,7).

The impact of food allergy on children and their families is substantial, involving safe dietary substitution of growth-critical foods, food safety awareness and the availability and confidence in use of rescue medications, including adrenaline injections. Cow's milk allergy is considered one of the most prevalent food allergic diseases in children. There are major direct and indirect impacts of CMPA, not only for the patient and the family, but also a substantial economic burden on the health system (8).

1.2 Clinical manifestations of CMPA

CMPA is the first of the allergies to be diagnosed in an infant, usually starting with the introduction of infant cow's milk-based formula. In about 60% of cases, it manifests after the first intake and rarely initiates after the first week of formula introduction. Occasionally, it also appears after skin contact and even kisses with someone who has been in contact with milk. CMPA can even present if the patient is exclusively breastfed (9).

1.2.1 Types of allergic reaction

Immediate allergic reaction

Allergic simptoms appear from minutes to two hours after exposure to cow's milk. It usually presents as IgE mediated sensitization (positive Skin Prick Tests (SPT) and/or positive specific IgE) with involvement of the skin (70-75%), abdomen (13-34%) and respiratory reactions (1-8%). You could also see more than one affected organ (26%) and even anaphylaxis (1-4%) (10).

Major symptoms include:

- Anaphylaxis. The most severe CMPA manifestation. Usually associated with early skin manifestations (local or generalized urticaria, angioedema) digestive (oral allergy syndrome, abdominal pain, vomits or diarrhoea), respiratory (80% of the cases: dyspnoea, bronchospasm, stridor, hypoxemia), cardiovascular (20% could have hypotension, syncope or shock) and neurologic (tremors, confusion, seizures and syncope) symptoms (9–11).
- Gastrointestinal manifestations: CMPA could have symptoms from the mouth to distal lower intestines. It could present as rejection to baby bottle associated with crying and irritability, with no other clinical manifestations. Other presentations include an oral allergy syndrome (OAS) which includes lip and tongue angioedema, oral pruritus and swallowing complaints after the ingestion of milk. Stomach and small intestine symptoms will manifest

as nausea, vomits and colic-like abdominal pain. Large intestinal symptoms manifest as abdominal pain, diarrhoea, and occasionally bloody stools (9–11).

- Respiratory Symptoms: Usually presented together with other systemic manifestations. They can present as asthma or rhinitis secondary to cow's milk ingestion. Usually associates with severe allergic reaction. Inhalation of boiled cow's milk fumes could also create severe respiratory symptoms (9–11).
- Cutaneous symptoms: Most frequent manifestations. Includes erythema with or without acute urticaria or angioedema (9–11).

Delayed reactions

Also called non-IgE reactions (negative SPT and specific IgE for milk). Symptoms appear usually after 2 hours of milk ingestion. Digestive symptoms are the most common type.

For the purpose of this research project, we will refer as CMPA only as IgE-mediated CMPA (immediate allergic reaction).

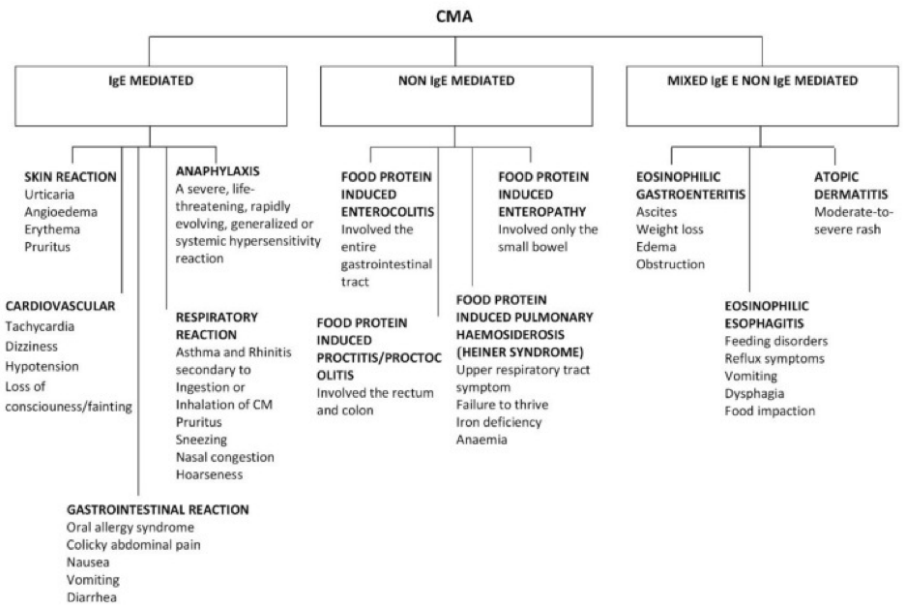


Figure 1 *Clinical presentation of cow's milk protein allergy CMA: Cow's milk allergy (Figure taken with permission of Gianetti et al.) (11)*

1.3 Allergens

Allergens are substances, proteins in most cases, that induce IgE responses (first a sensitization phase and a clinical response in subsequent exposures) (12).The subsequent response needs the binding of an allergen to IgE receptors.

1.3.1 Major and relevant minor allergenic molecules

We defined major and minor allergens depending on the IgE-binding frequency classifying it as a major (> 50% IgE-binding) or minor (<50% IgE binding) (13).

In the case of cow's milk, it contains 30-35g of proteins per litre. These proteins are divided in 2 fractions: curd (coagulum) with 80% of CM proteins and whey (lactoserum) with 20% of proteins. The major allergens of CM are casein, betalactoglobuline and alpha-lactalbumin. CMPA patients are usually poli-sensitized to different allergens. (See Table 1) (14).

Table 1. *Major and minor cow´s milk (CM) allergens, sensitization and cross reactivity (figure taken with permission) (14)*

Allergen name	Allergenicity	Sensitization rate % among those reactive to CM	Laboratory cross-reactivity	Clinical cross-reactivity
Curd fraction (coagulum)				
Caseins (Bos d 8)	Major	63	>85% with sheep and goat milk caseins	>90% with other mammalian milks (27) 20% with mare´s milk 29 and donkey milk 30
Alpha $_{s1}$ -casein (Bos d 9)	Major	98*		
Alpha $_{s2}$ -casein (Bos d 10)	Major	94*		
Beta -casein Bos d 11)	Major	91*		
Kappa-casein (Bos d 12)	Major	91*		
Whey fraction (lactoserum)				
Alpha-lactalbumin (Bos d 4)	Major	51	80% with beef	15-20% with raw beef
Beta-lactoglobulin (Bos d 5)	Major	61		
Bovine serum albumin (Bos d 6)	Minor	43		
Inmunoglobulins (Bos d 7)	Minor	36		
Lactoferrin	Minor	35		

1.3.2 Heat stability of major allergens and clinical relevance

Whey proteins (alpha-lactalbumin and beta-lactoglobulin) are heat-labile, therefore the baking milk will affect the allergenicity of these CM proteins, whereas casein (curd) has been more resistant to heating compared to whey proteins (11).

The implication of this mechanism in management of CMPA will be explained in greater detail later.

1.4 CMPA Resolution

There is no unified treatment of CMPA. Traditionally, it was thought to be a transient allergy with a high rate of resolution in childhood. However, the resolution rate is not heterogenous across different studies. Different resolution rates depending on the study are shown in Table 2 (11).

Table 2 *Natural history of CMA in different populations and settings (adapted from Gianetti et al.) (11)*

Author, Year	Number of Subjects	Population /Study Design	Tolerance Rate	Age of Tolerance
Host. et al., 2002	39 (24 IgE mediated)	General prospective birth control	56% 77% 87% 92% 97%	1 2 3 5 15
Vant. et al., 2004	162 (95 IgE-mediated)	Referral retrospective	44% 69% 77%	2 3 4
Garcia-Ara et al., 2004	66 IgE mediated	Referral retrospective	68%	4
Saarinen. et al. 2005	118 (75 IgE mediated)	General prospective birth cohort	51% 74% 85%	2 5 8.6
Skripak et al.2007	805 IgE mediated	Referral retrospective	19% 42% 64% 79%	4 8 12 16
Fiocchi et al., 2008	112 IgE mediated	Referral retrospective	52.7%	5
Martorell et al., 2008	112 IgE mediated	Referral retrospective	82%	4
Santos. et al., 2010	170 IgE mediated	Referral retrospective	41%	2
Ahrens. et al., 2012	52 IgE mediated	Referral retrospective	61.5%	12
Elizur. et al., 2012	54 IgE mediated	General prospective birth cohort	57.4% 65%	2 4
Wood F. et al.2013	293 IgE mediated	Prospective	53%	5.5
Yavuz, et al., 2020	148 IgE mediated	Prospective	20% 34% 39%	2 4 6
Schoemaker. et al., 2015	55	EuroPrevall, European population-based prospective	57%	2

1.5 Diagnosis of CMPA

Diagnosis of IgE-mediated CMPA is made on the basis of a compatible history, including improvement after avoidance and the presence of a positive diagnostic test using skin prick test or specific IgE. The oral food challenge is the gold standard for diagnosis of CMPA. However, in children less than 1 year old, it is a common practice to make a diagnosis with allergy tests (skin prick test and/or Specific IgE) and with a focused clinical history that describes IgE symptoms (within the last 3 months). The diagnostic oral food challenge is selected only for doubtful cases (15) several guidelines have argued over the use of oral food challenge (OFC) to diagnose CMPA(16). The Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guidelines from the World Allergy Organization (WAO) highlighted the necessity of Oral Food Challenges in the initial diagnosis (17). Meanwhile, other societies recommend against its use, due to its lack of practicality. The British Society of Allergy and Clinical immunology (BSACI), for example, highlighted no need of OFC in patients with a wheal size of cow's milk prick test > 6mm in children less than 2 years old and >8 in children older with a 100% specific positive challenge (15,18). From the empirical and real-life settings view in allergy clinics, the use of a clinical history and positive values of skin prick testing or specific IgE is considered sufficient for diagnosis of IgE-mediated CMPA (5).

Even though molecular allergy is considered a useful tool for monitoring natural tolerance and reactivity to baked milk, it is not a recommendation for the standard evaluation of CMPA. The use of cut off points off the skin prick test and specific IgE to determine the decision making of the reintroduction of milk is still a normal practice in patients that are treated with strict avoidance of milk using these parameters to decide the moment of reintroduction of milk with an oral food challenge procedure (see Table 3) (14).

Table 3 *Proposed specific IgE decision point for CMPA diagnosis (14)*

OFC to regular (non-heated milk)		
	SPT to CM, mean wheal diameter (mm)	**CM-IgE [kUA/L]**
Defer >95% PPV	>8	>15
		> 5 if less than 1 year old 17
Perform <50% PPV	Not done	<5
OFC to baked milk		
	SPT to CM, mean wheal diameter (mm)	**CM-sIgE [kUA/L]**
Defer >95% PPV	Not done	>24.5
Perform >90 NPV	<12	<9.97
	SPT to Casein, mean wheal diameter (mm)	**Casein-sIgE [kUA/L]**
Defer >95% PPV	Not done	10
Perform >90 NPV <50% PPV	<9	<5

1.6 Prevention of CMPA

Several strategies have been introduced in the prevention of CMPA, usually divided in three categories:

- Primary prevention of initial IgE sensitization which involves the understanding of the mechanism of development of CMPA. Several factors are involved in this, which include allergy related family history, environmental factors, maternal diet during pregnancy and, of course, the involvement of breastfeeding (Figure 2)(19).
- Secondary prevention of the disease progression from mild to moderate to severe symptoms or another allergy phenotype (20). The strategies include the use of extensive hydrolysed formula (eHF), partially hydrolysed formula (pHF), amino acid-based formula and modulation of the microbiome.
- Tertiary prevention in children with established CMPA is based on avoidance off allergenic food and treatments that target tolerance induction (21).This will be addressed in the next chapter.

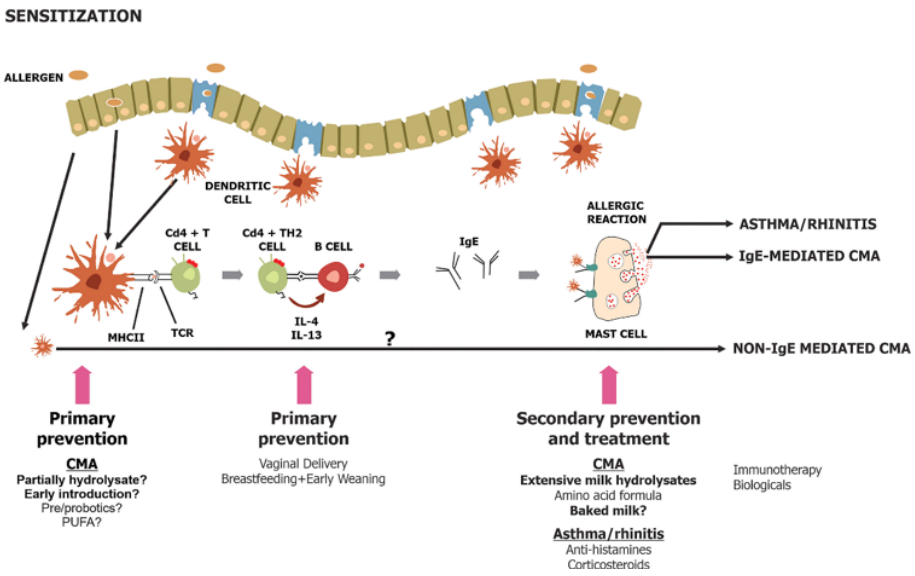


Figure 2. *Schematic diagram on primary, secondary and tertiary prevention in cow's milk protein allergy. CMA: Cow's milk Allergy, PUFA: Polyunsaturated Fatty Acid, IL: Interleukin, TCR:T-cell receptor, MHCII: major histocompatibility complex II (19)*

1.7 Treatment of CMPA

1.7.1 Strict Avoidance of CM and derivatives

Complete avoidance of milk protein is still the usual treatment in several countries. When possible, it is advised to continue with breast milk, or a substitution with a specialized hypoallergenic formula (hydrolysed or amino acid-based formulas) if breastfeeding is not available (22).

To avoid the persistent symptoms, the elimination diet must be effective and complete. The usual strategy includes information and support of a dietician to explain the most accepted foods and possible substitutes.

The periodic follow up of patients is necessary to avoid prolonged use of a strict avoidance diet (18).

Periodic re-evaluations every 6-12 months with laboratory testing and oral food challenges are recommended. A drop in CM specific IgE by 50% or more in 1-2 years is a favourable prognostic indicator of natural tolerance (23). This approach will often result in an exclusion of milk products until 2-5 years of age (24).

Despite the labelling of food with proper indications being mandatory by law in most countries, including the EU, accidental exposure still occurs. Contamination of food in restaurants, canteens and other settings are possible. One study followed 80 patients with CMPA until the achievement of tolerance or up to the age of 18 years finding accidental ingestion of milk in at least a third of them (25).

Even though that milk avoidance is the usual first-line treatment across several countries, in the last decade its use is no longer as widely accepted as it was before. For instance, there is evidence that children at their first year of life have limited success in the reduction of food sensitization and food allergy with and milk avoidance treatment (21).

1.7.2 Role of baked milk and the use of food ladder/ home re-introduction milk strategy

As discussed previously, the baking process alters the structure of different milk allergens changing its stability and subsequently creating a decreased allergenicity (decreased IgE binding) (11). In many cases, this is because of the destruction of conformational epitopes (antigenic determinant that binds with the IgE receptor) of milk proteins. Children with transient milk allergy are considered likely candidates to tolerate baked-milk products (26). Several studies reported good tolerance to baked milk introduction in children, even reporting to accelerate tolerance to fresh milk (27,28). This is supported by the observation that an increase in the intensity of IgG4 binding to CM epitopes occurred concurrently with a decrease in IgE-binding intensity among patients who recovered early from CMPA. The production of IgG4 induces tolerance by blocking the binding of specific IgE to allergen (22). Therefore, it can be hypothesized that the gradual introduction of denatured epitopes of baked milk proteins promotes the production of IgG4, thus inducing tolerance in milk allergic patients (Figure 3).

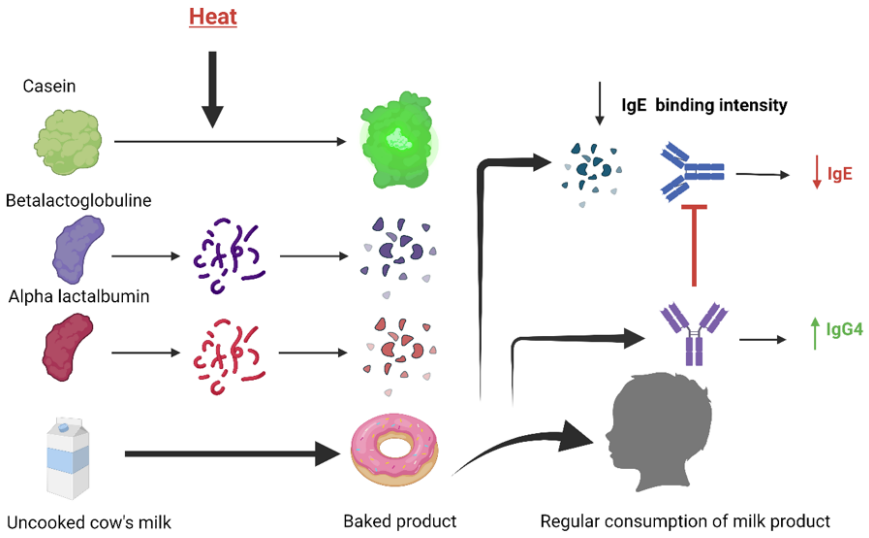


Figure 3. *Proposed mechanism of the baked milk tolerance acceleration*

Trying to develop a successful reintroduction of milk protein, or in some cases tolerance of milk (definition depends on the literature) could be achieved by using two different approaches. The first option might be with the introduction of food that contains baked milk such as pizza or muffins with the use of supervised oral food challenges (28). The second approach will be by the use of ladders, that in this context means a stepwise progression from extensively heated to less heated food. This strategy was primarily used in the treatment of non-IgE mediated milk allergy only, however their use in IgE-mediated allergy has increased as surveys have demonstrated (29).

The first published ladder was the Milk Allergy Primary (MAP) guideline in 2013, indicated for mild to moderate non-IgE mediated cow's milk allergy. This is a 12-step approach focusing on typical British foods. An international (IMAP) version was published in 2017 (shortened and improved). A complete strategy and guideline using a milk ladder for IgE mediated CMPA is not yet established. However, its empirical use in several countries has increased in the last decade. A 2017 publication showed that 60% of surveyed physicians acknowledged using the MAP and the IMAP ladder for IgE-mediated allergies (29). With the introduction of this ladder, other allergy societies (like the Canada allergy society) have decided to adapt and create their own ladders (30).

One research study published about the use in IgE-mediated disease was done by Ball et al. in the United Kingdom (31). This is a real-life retrospective study of the use of an adapted milk ladder management for CMPA IgE mediated (see Figure 4). The strategy uses four stages that starts from baked milk product (biscuit) with increases in different amounts, with a progression of volume and levels of baked milk through all stages. In Stage-4, the products are uncooked and finished the stage with the introduction of milk. They considered an official 4+2 months follow up to progress to the next stage. The study retrospectively recollected information of 86 patients, with only 8 patients not achieving toleration of all dairy products, 43% of them presented some sort of allergic reaction during the management, with no anaphylaxis diagnosis that required intramuscular adrenaline. The authors highlighted the good compliance of the patients, with

very few episodes of accidental or inappropriate diet exposure. Their approach during this milk strategy included the use of not only baked milk products, but the necessity of low doses of it during the first stage. This was a process of approximately 5 weeks, with a first introduction of malted biscuit product. The beginning phase starts with the ingestion only of a small crumb per day for a week (about 0.35mg of milk protein), and then increasing the amount until a total of between 23 to 43mg of milk protein is ingested (31). The most interesting part of this study comes not only from the achievement of full reintroduction of milk at this age, but the idea of having a safe and successful home-based introduction strategy for IgE-mediated CMPA. Finally, the study discussed an important thought: Are we just using a new method of introduction of milk for patients that already have self-resolved their IgE mediated CMPA due to the natural course of the disease? Or is this also a method of oral immunotherapy to induce tolerance in someone who would not develop tolerance by themselves? The only way to even have some sort of answer would be with further studies that compare different strategies of milk reintroduction, as our study aimes to do. For now, the term "home reintroduction of milk" would be more suitable as it does not suggest that this is a new method of oral immunotherapy (OIT), but a different treatment strategy for the management of IgE mediated CMPA. OIT will be explained later doing this manuscript.

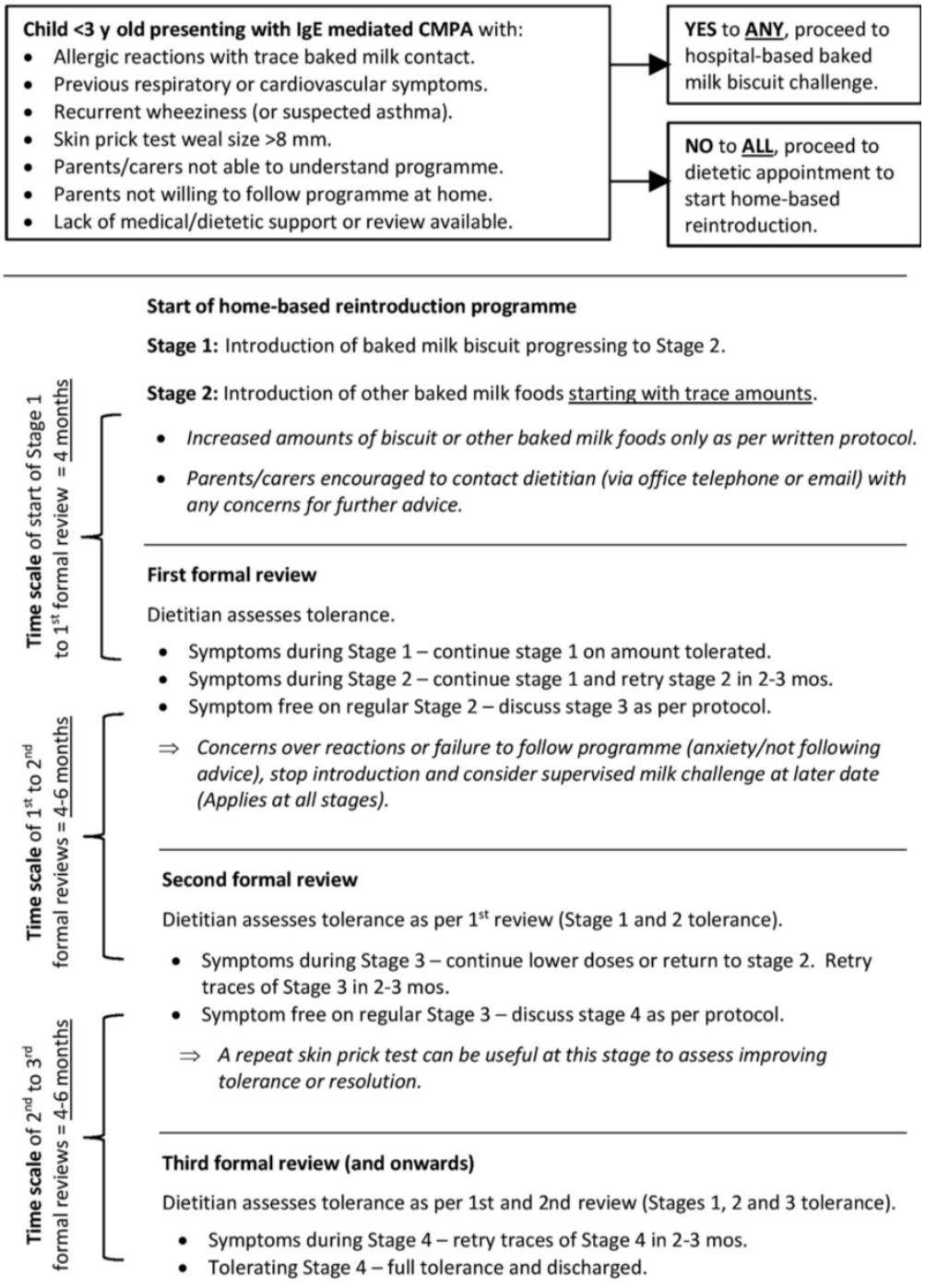


Figure 4 *Management of home-based Milk introduction cow's milk protein allergy (CMPA) IgE mediated (31)*

The latest study to evaluate the use of the milk ladder as a method of home introduction to milk for those with IgE-mediated CMPA was conducted by d'Art et al. published in April 2022 (5). This was a randomized trial to evaluate the progression through the milk ladder of infants who received a single dose of the elicited dose of milk (ED05) compared to the control group who did not receive a single dose of ED05, this is also the first published prospective study on the effectiveness of the MAP milk ladder for infants with IgE mediated CMPA. Unlike previously described trials, this study excluded children who were already tolerating baked milk.

They concluded that the very act of giving infants a single low dose of cow's milk in the presence of their mothers promoted parental confidence in home introduction, leading to accelerated progression up the milk ladder. This is supported by previous single dose studies of ED05 of peanut and milk (32). While it was to be expected that some children would have mild symptoms when transitioning to a higher step on the milk ladder, no serious or unexpected adverse reactions occurred in children progressing through the ladder. Three accidental exposures to milk occurred over the course of the study, all of these happening outside the home in childcare settings and relatives' houses (5).

Due to the limited efficacy of the avoidance diet, new ways to treat food allergy emerged this will contribute with the changes in the way we approach CMPA and food allergy in general (11).

1.7.3 Oral Immunotherapy

Oral Immunotherapy (OIT) involves the mixing of allergenic food into a vehicle and consuming it in gradually increasing doses (33). This could prevent allergic symptoms and reduce the risk of severe anaphylactic reaction. This progressive increase of doses (usually increasing the milligrams of proteins per dose) aims to reduce allergic symptoms in accidental exposures. In other words, attaining desensitization and, if possible, permanent tolerance (nowadays it is preferable to use the term "un-sustained response") of the culprit food.

In summary, oral desensitization is a reversible state of a non-allergic response after gradual introduction of the food allergen. This state could disappear after sudden avoidance of the allergen. In the case of tolerance, the state is permanent, and the allergic symptoms won't reappear if we decide not to ingest the culprit food regularly (34). From the particular point of view of allergy research, the term un-sustained response is preferable until a full tolerance result is achieved (that includes observation of the patient response the food for several years). On this manuscript, these two terms are used as synonyms because research literature uses these terms similarly, and the difference will be usually due to the year of publication of the study (Figure 5).

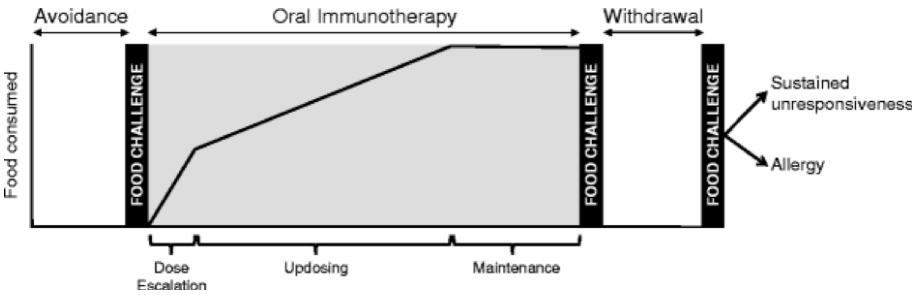


Figure 5 *Typical approach to food oral immunotherapy*

Mechanism of OIT

Despite the use of OIT being reported for the first time in animals in 1909 (35), the exact immunological mechanism is still not fully understood. However, it is already known that it reduces the activation of mast cell and basophil mediators. It also increases the levels of specific IgG_4, decreases levels of specific IgE, activates the pathways of specific T regulatory cells and inhibits the response of T helper 2 cells (TH2)(36).This proposed mechanism and other mediators can be seen in Figure 6.

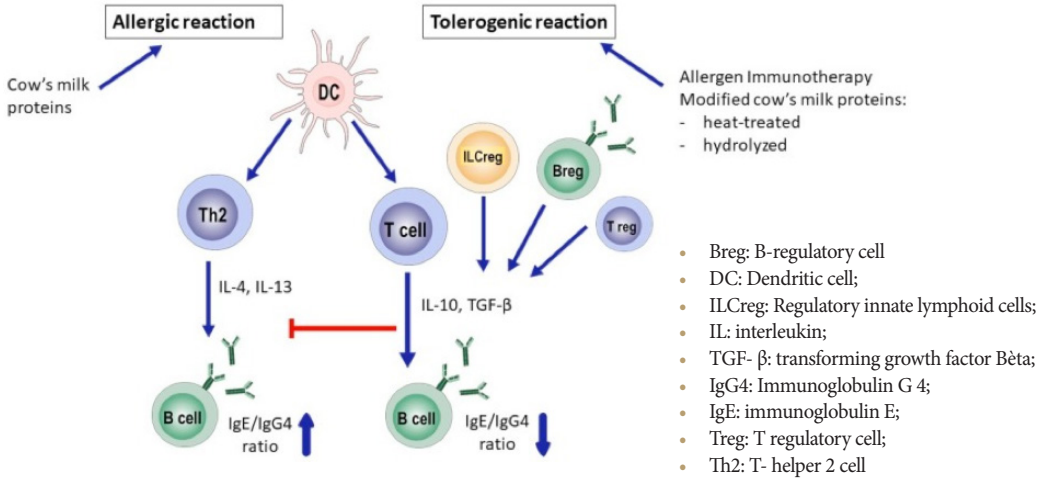


Figure 6 *Proposed mechanism of Immune response in the allergic versus tolerant state for cow's milk proteins (37)*

OIT in CMPA

At the moment, OIT is potentially indicated in CMPA patients unable to achieve positive results with milk avoidance. The European Academy of Allergy and Clinical Immunology (EAACI) recommends OIT for CMPA in children around 4-5 years of age in order to increase the threshold of reaction while on treatment in children with persistent CMPA (38) Even though it is used widely in many different countries, a standardized protocol for OIT hasn't been established yet, mostly due to the different methods that are established differ in timing, doses and type of food (heated or not) (39).

Perhaps because of these differences in the methodology, the results of the different clinical trials (Table 4) differed in the effectiveness of oral immunotherapy in CMPA and the convenience of OIT for partial or total tolerance of milk in these patients. For this reason, EAACI has not given a recommendation yet (36).

Table 4. *Efficacy oral immunotherapy adapted from Nutrients 2021, 13, 1525 (11)*

Author, Year	Type of Study	Type of Milk	Population (n)	Age (years)	Partial Tolerance	Complete Tolerance
Meglio P. et al., 2004	Open-label	Fresh CM	21	6-10	14.3% (40-80 mL of CM)	71.4 (200mL CM)
Narisety SD. et al., 2009	Open	Fresh CM	15	6-16		33% (16g of CM protein)
Goldberg M. et al., 2015	Open	Baked CM	14	6.5-12.7		21% (1.3 g of CM proteins)
Takahashi M. et al. 2016	Open	Microwave heated CM	31	5-17		45.2% (200mL of CM)
Ebrahimi M. et al.2017	Open	Fresh CM	14	3.5-7		92.9 (200-250 mL CM)
Skripak et al., 2008	Randomized, double-blind, placebo-controlled	Fresh CM	13	6-17		30.8 (500mg of CM proteins)
Longo G. et al., 2008	Randomized open label	Fresh CM	30	5-17	54% (5-150mL CM)	36% (>150mL CM)
Pajno GB. et al., 2010	Randomized, placebo controlled	Fresh CM	15	4-10		67% (200mL CM)
Martorell A. et al., 2011	Randomized, placebo controlled	Fresh CM	30	2-3		90% (200 mL CM)
Amat F. et al.2017	Randomized	Baked CM Fresh CM	43	3-10	26.8% (0.27-2.5g CM protein)	36.6% (2.72g CM proteins)

Author, Year	Type of Study	Type of Milk	Population (n)	Age (years)	Partial Tolerance	Complete Tolerance
Maeda M, et al., 2020	Randomized controlled	Fresh CM	28	3-12		50% (100mL CM)
Mota I. et al., 2018	Prospective	Fresh CM	42	2-18		92% (200mL CM)
Berti I. et al., 2019	Prospective	Fresh CM	73	3-11		97% (150mL CM)
De Schryver S. et al., 2019	Prospective, randomized-controlled	Fresh CM	41	6-18		73.2 (200mL CM)
Efron A. et al.,2018	Retrospective, case-control	Fresh CM	43	1-4		86% (250mL CM)
Kauppila T.K. et al., 2019	Retrospective	Fresh CM	296	5-17		56% (200mL CM)
Demir E. et al., 2020	Retrospective, cohort study	Fresh CM	47	3-13		89.3% (200mL of CM)
Gruzelle V. et al., 2020	Retrospective	Baked CM	64	2-16		42.2% (254mL CM)

1.7.4 Early introduction of milk using an OIT strategy in young infants

As described, the use of CMPA OIT is commonly started in patients between 4-6 years, and very little research has been done in patients less than one year old. The first introduction of this type of management was in 2011 by Reche et al. which did a case control study of 20 patients comparing milk avoidance and an OIT protocol, with a one-year follow up. After this period, all OIT patients were tolerant to milk in comparison to 3 that were tolerant in the control group ($p=0.003$) (40). Following the same pathway, three more studies have investigated the use of early introduction OIT in young infants, with similar results (no severe adverse effects, high rate of compliances, average time of 6 months) as explained in Table 5 (41–43).

Table 5 *Early introduction of milk in young infants less than 1 year published articles*

Author, Year	Type of Study	CMPA diagnosis	Type of Milk	Population (n)	Age (months)	Completed OIT months)	Complete Tolerance	Adverse reactions (Type)	Intramuscular Adrenaline use
Reche et al 2011 (40)	Case-control study	Clinical history and SPT/ Sp IGE	Fresh CM	20 (10 case group, 10 control group)	Less than 12 months	5.3	100% (10)	4 mild	No
Ji-HyukL et al 2013 (41)	open label Randomized control	DBPCFC	Fresh CM	26 (14 OIT group 12 control group)	7-12 month	6	100% (14)	12 mild	No
BertiI et al 2019 (42)	Prospective Home introduction	Clinical history and SPT/ Sp IGE	Fresh CM	68	3-11 months	5.5	97% (66)	29 mild-moderate	No
Boné et al 2020 (43)	Retrospective Cohort Study	Clinical History and SPT/Sp IgE	Fresh CM	335	Less 12 months	3.6	98.2% (329)	136 122 mild 14 moderate	No

Discrepancies in the structure of the research

The main controversial point about the use of this type of management is the outcome. It is not yet clear if it is used as a way to achieve tolerance or desensitization. Two studies decided to include patients and diagnose them with CMPA without the use of a food challenge (41,43). One of them used an open food challenge protocol to establish the diagnosis (42). The reason for this incongruency relies on presumption that, although the main diagnosis of a food allergy is an oral food challenge, due to its impracticality, the investigators decided to use a clinical diagnostic approach in most of the patients. Another substantial issue is that there is not an available way to verify the achievement of tolerance. Due to the age of the patient and again the impracticality of the procedure, all the participants that passed the OIT treatment continued eating cow's milk products without any washout period and re-introduction using and OFC.

Is it an OIT or a reintroduction management?

Finally, the main point of discussion is if the patients were going to achieve a spontaneous resolution of CMPA disease with or without this treatment. The three studies that have considered this subject concurred that it is too early to know and that further long-term data and new research are needed to answer those questions (41–43).

For the purpose of this study the use of OIT in young infants less than one year was not considered an immune tolerance strategy due to the lack of evidence. However, it was considered a useful tool of reintroduction of milk in patients.

1.7.5 General view of the evolution in the treatment of CMPA

With this evolution of treatment strategies, the active role of caregivers and healthcare professionals in the management of CMPA has changed our main responsibilities from passive observer to an active promoter of milk reintroduction and tolerance. A summary of the milestones in the development of treatment for CMPA is shown in Table 6 and Figure 7.

Table 6: *Milestones of the development of treatment of CMPA*

1909	First recorded use of OIT in animals	(35)
2001	Allergenic IgE and IgG antibodies for b- and k-casein and alpha(s1)-casein epitopes were identified, with higher levels of these IgE antibodies associated with persistent CMPA.	(44–47)
2002	A significant number of Infants who consume AAF achieve tolerance by 20.5 months	(48)
2004	Children aged 6 years or more with severe CMPA found to be tolerating a daily intake of cow's milk following 6 month OIT protocol	(49)
2008	Significantly smaller SPT mean wheal diameters and significantly greater casein-IgG4 concentrations were shown in CMPA patients who ingested baked milk products for1 3 months.	(27)
2011	Subjects who incorporated dietary baked-milk were more likely to become unheated milk-tolerant. Infants with mean age of 3 months with CMPA who underwent an OIT protocol became tolerant to milk	(26) (40)
2013	Publication of Milk Allergy Primary (MAP) guideline in 2013 for non-IgE mediated cow's milk allergy	(50)
2017	International version of milk ladder (iMAP) published Consumption of Lactobacillus rhamnosus probiotic with eHCF promotes tolerance to cow's milk	(51) (52)
2018	Clinical trial evidence showing improved tolerance to fresh milk following baked milk introduction compared to strict avoidance.	(28,53,54)
2019	First study to assess the effectiveness of the milk ladder in IgE mediated CMPA, with most children completing the ladder and tolerating almost all dairy products.	(31)
2020	Large trial in infants under 1 year showing increased tolerance to cow's milk following OIT protocol	(43)
2022	Parental anxiety correlates with progression through the milk ladder	(5,31)

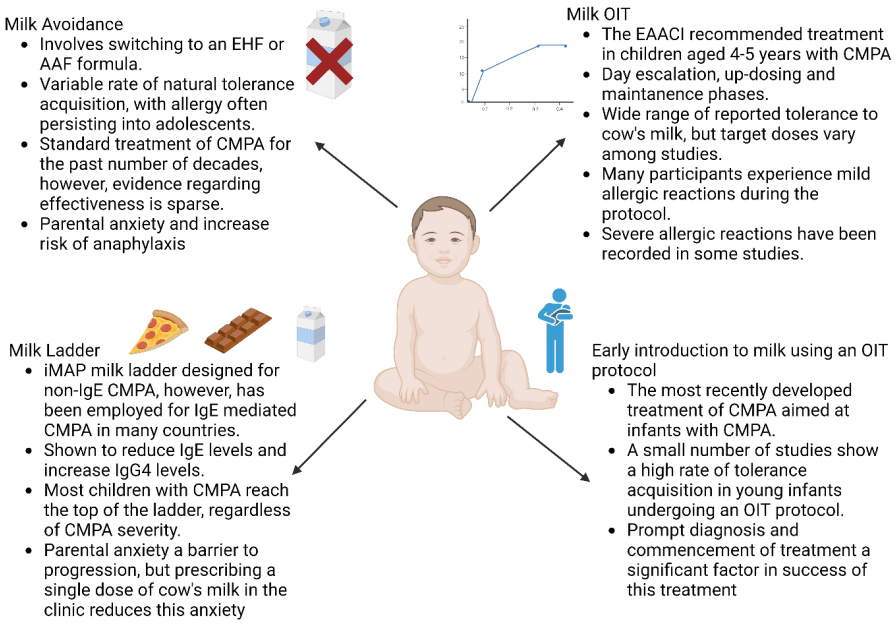


Figure 7. *Summary of CMPA treatments*

1.8 The situation of allergy and differences in management in two European countries

National health systems across Europe widely differ in terms of allergy services provided and access to specialised healthcare professionals. In addition, recent statistics revealed a deficit and growing disparity in the number of allergists in Europe (55).

The promotion of good patient care requires insight into the current state of allergy services across Europe. Identification of barriers and opportunities are needed to improve health care provisions (56).

As seen in Figure 8, the difference in the amount of allergy centres across EU countries differs substantially. For the purpose of this study, we focused on the management strategies of CMPA in two different countries: Spain and The Republic of Ireland.

1.8.1 Ireland

Nowadays, allergic diseases are treated in two main cities in Ireland: Dublin and Cork, with only four paediatric allergists (3 in Dublin and 1 in Cork) across the country. Fifteen local hospitals treat patients with allergies, with only two having resources for allergy care (prick test and oral challenges)(57). There is no adult allergist in Ireland. However, the clinical immunologists across the country takes care of allergy diseases in the adult population (58).

Several complexities of allergic diseases are treated in the two allergy centres. (57) However, due to the increased number of patients, waiting lists (in 2020 routine waiting list was longer than 18 month in both cities) and the lack of human resources, the care of the patient is severely jeopardized.

1.8.2 Spain

Spain has 2 types of Allergy schemes: Allergy and clinical immunologist scheme (4-year training program) and paediatric allergist scheme (trained in paediatric sand subsequently 2-year allergy training). The latter doesn't have an official recognition by the Spanish Health Care System, although it has the accreditation of the European Training Committee – Paediatric Allergology (ETC-PA), a working group of the Paediatric Section of EAACI and the European Union of Medical Specialists (UEMS), section of paediatrics (59). The usual coverage of allergists in Spain is not well distributed across all the communities, however it is totally consolidated in the country with a quality training scheme (60).

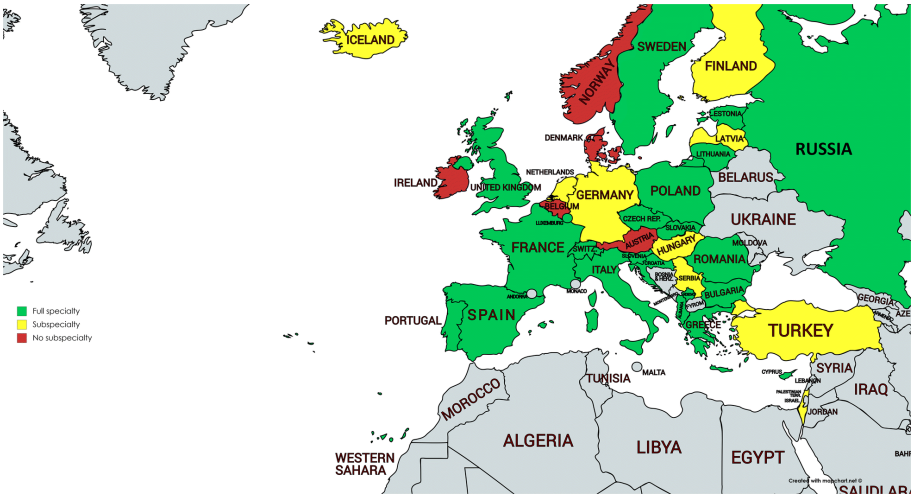


Figure 8 *Overview of countries with a specialty (green), subspecialty (yellow) or without a (sub)specialty (red) in EU (56).*

The possibility of a fast-track referral and subsequently a brief return or follow up is crucial for allergy patients. Children with food allergy need to have appropriate access to a medical expert and diagnostic support. Since the level of uncertainty about future reactions is a major concern for patients, an appropriate use of the services, with better protocols and adequate management of the common allergy diseases should be a priority (61).

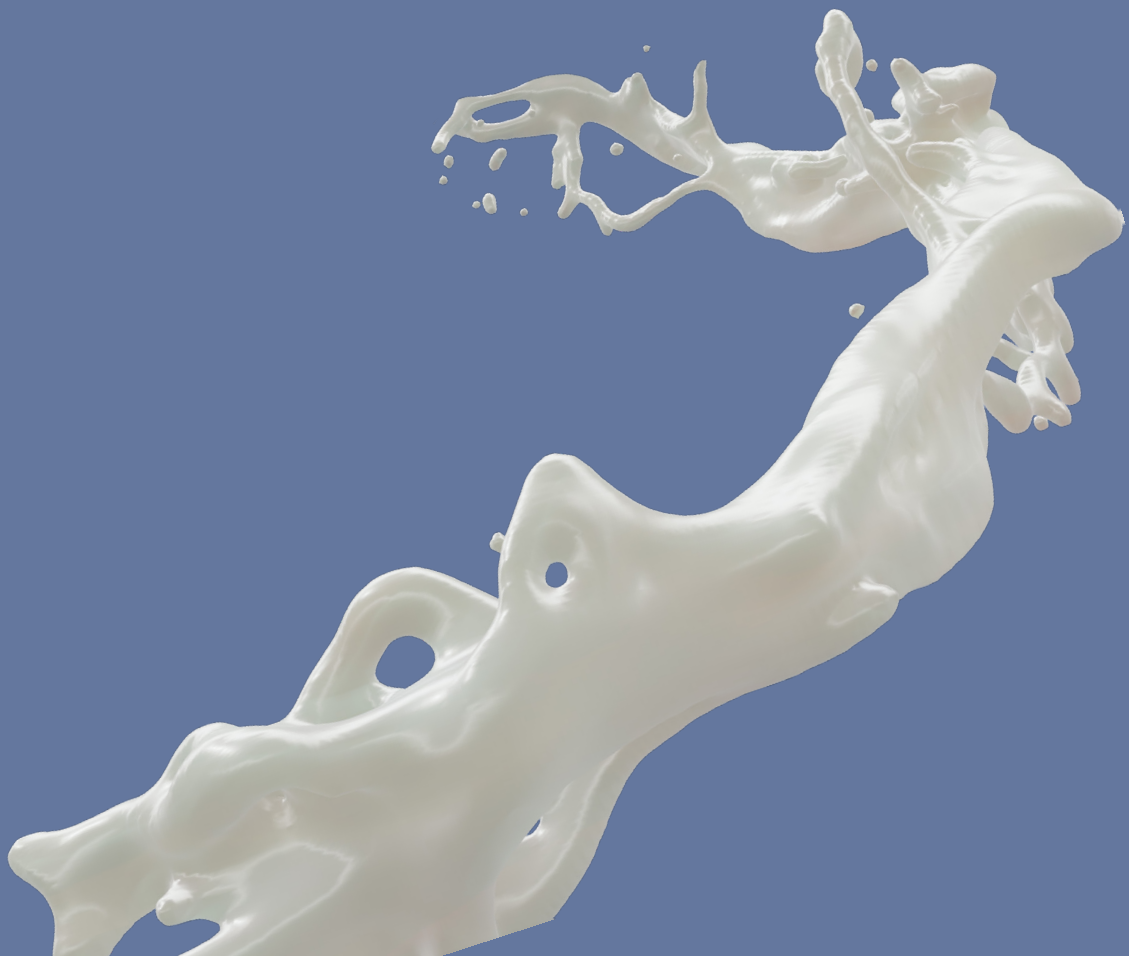
1.8.3 Difference in management

As every country has its own guidelines, differences in the management of CMPA are common. Being a paediatrician that has been working in both countries for more than 11 years (>2 years in Ireland and 9 years in Spain), different strategies to treat CMPA are one of the reasons to develop this study. On one hand, the use of strict avoidance of milk is the most common approach in Spain. The use of OIT as a second line of treatment if the patient does not have a natural tolerance after milk avoidance is used in a tertiary level of allergy centres. However, there is only one centre, Hospital Miguel Servet in Zaragoza, which is using an early introduction of milk protocol in young infants less than one year old as a routine treatment for CMPA. To the best of our knowledge, no hospital in Spain uses home-based introduction of milk using milk ladder for IgE mediated CMPA patients.

On the other hand, the allergy community in Ireland is using the home introduction of milk, also known as milk ladder approach as the first option in children with CMPA.

This research study tried to evaluate the use of different strategies for treating CMPA, we hope that this study will enlighten us about more than one way to treat a disease that is increasing every year (62). Our main purpose with this study is to create discussion, not only about the benefit of one strategy over another, but also to appreciate that there is more than one way to treat a disease taking in consideration the reality of the healthcare system of each country, in this case Spain and Ireland.

2. OBJECTIVES AND ENDPOINTS



2 Objectives and Endpoints

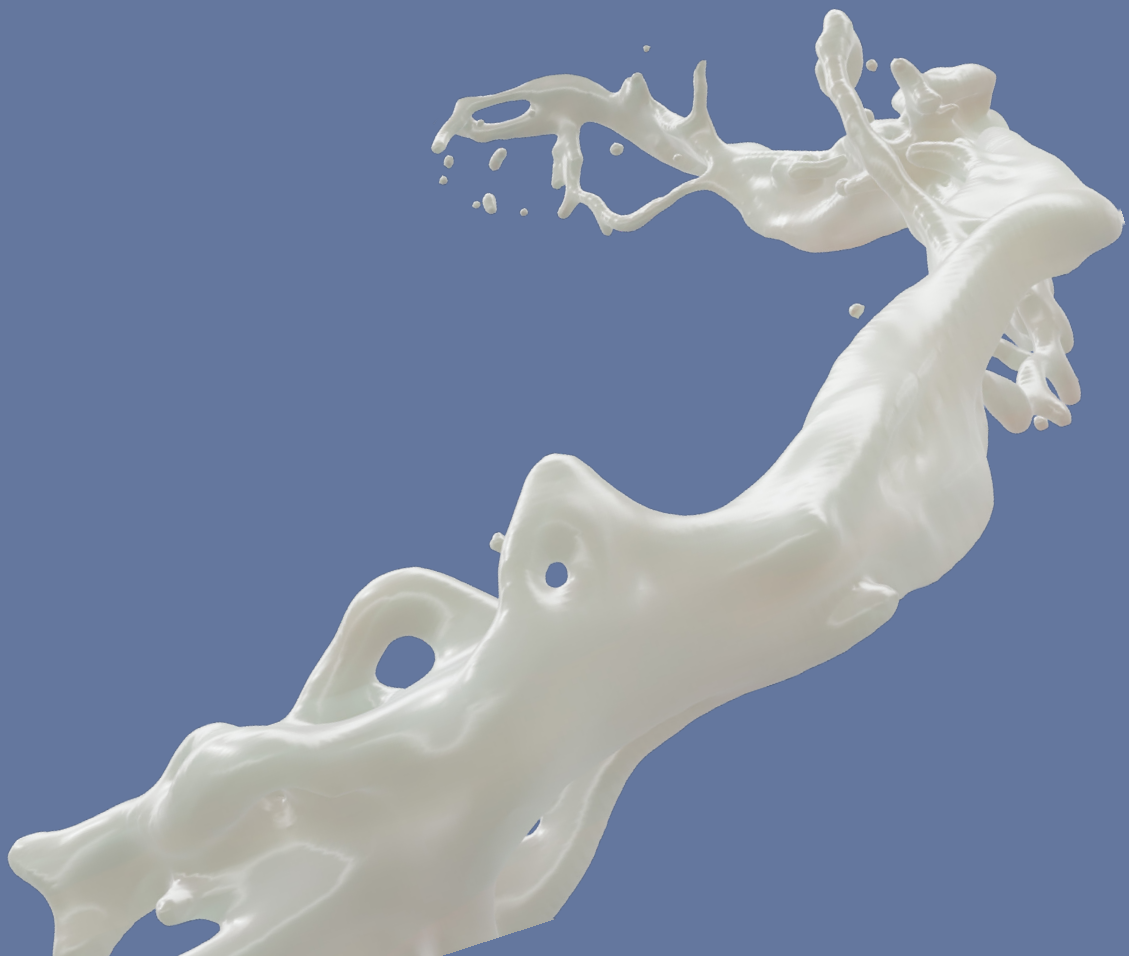
2.1 Primary objective

To compare the rate of the full reintroduction of milk of three different IgE-mediated CMPA treatment strategies. The main outcome was the success rate in full reintroduction of milk, which was defined as the intake of more than 150ml of cow's milk or the equivalent intake of 4.5g of milk protein daily without any symptomatology.

2.2 Secondary objectives

- To analyse the influence of other allergic symptomatology in the outcome of the three treatments in CMPA IgE-mediated.
- To analyse the influence of other food allergies in the outcome of the three treatments in CMPA IgE-mediated.
- To conduct an analysis of immunological values in the three cohorts.

3. MATERIALS AND METHODS



3 Materials and methods

3.1 Study design

This was a multicentric retrospective analysis of three cohorts of patients who received different managements of IgE-mediated cow's milk protein allergy. It consisted of retrospectively analysing charts from 200 patients diagnosed with IgE-mediated CMPA between 2011 and 2020 in each of the following centres as described in Figure 9:

- The Paediatric Allergy Service of Cork University Hospital and from The Weir Family Health Clinic, both in Cork, Ireland.
- The Paediatric Allergy Unit of Hospital Universitario Miguel Servet, in Zaragoza, Spain.
- The Paediatric Allergy Unit of Hospital Universitario de Móstoles, in Móstoles, Spain.

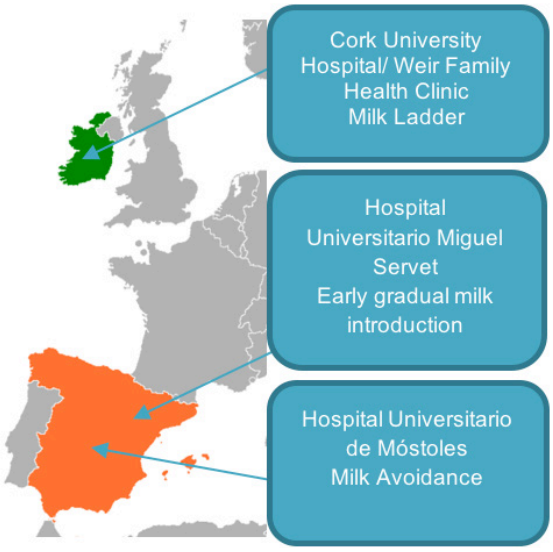


Figure 9. *Map of the research site and milk strategy*

3.2 Definitions

We defined CMPA as a patient with an immediate (less than 2 hours) symptoms after taking infant formula with a skin prick test ≥ 3 mm or a Milk specific IgE blood value ≥0.35 UI/ml. CMPA symptoms (also known as IgE mediated symptoms) include:

- Cutaneous: Urticaria and angioedema.
- Gastrointestinal: Vomits, abdominal pain.
- Respiratory: Posterior and anterior rhinorrea, eye irritation, eye or nose itchiness, cough, wheezing, sore throat, respiratory distress.
- Anaphylaxis: Rapid on-set systemic severe life-threating allergic reaction that needs to be treated right away, involving 2 or more systems that include airway, breathing, or circulatory problems and is usually, although not always, associated with skin and mucosal changes.

3.3 Management in each centre

All infants diagnosed for IgE-mediated CMPA were considered suitable for home milk reintroduction using the milk ladder escalation, strict milk avoidance or early gradual milk introduction. All the management strategies required that the parents/caregivers were able to understand the programme and be willing to follow it at home. None of the centres performed a confirmatory oral food challenge as this is not a standard practice in the real-life settings (15,63). At their outpatient visit, the parents were provided with instructions on their strategies.

Each of the participant centres use one of the management strategies already described (see Table 7):

Table 7. *Research site by city, country (including paediatric population) and management strategy*

Research Site	City	Country	Paediatric allergy Service or Unit	Paediatric population of the area (less than 17 years)	Milk Strategy
Cork University Hospital/ Weir Family Health Clinic	Cork	Ireland	yes	121863	Home milk reintroduction "**Milk Ladder**"
Hospital Universitario Miguel Servet	Zaragoza	Spain	yes	147906	**Early gradual milk introduction**
Hospital Universitario de Móstoles	Mostoles	Spain	yes	33092	Strict **Milk avoidance**

In the following section, we are going to summarize the main characteristics of each management strategy.

3.3.1 Milk Ladder

General recommendations while starting the milk ladder included to first try the milk products in small quantities and increase it progressively (e.g., crumbs and then 1/4 of a portion). If the food is tolerated, they will be allowed to eat it normally. If the patient is unwell or with symptoms its vital to stop upscaling the ladder until full recovery.

The usual recommendation for caregivers of a patient that haven't started milk products is to start with crumbs of malted milk biscuits, then a quarter, a half and then a full malted milk biscuit in a 2–4-week period progressively. In the following step, the caregivers will introduce other foods that are baked and made with flour and contain milk. Different options like cake, muffins, bread products like croissants or waffles, products containing butter or pastry could be available. Further upscaling steps include the introduction of milk products that are heated at some level (for instance, cheese). A few examples would include pizza, pies, rice pudding, chowder (milk in soup), chocolate milk cookies, etc. Finally, uncooked pasteurized milk products will be introduced. The product will include uncooked cheese or non-fermented desserts (cheesecake, mousse, cream) and fresh milk (including milk shake and ice cream).

The training of caregivers is done by a health professional in Cork. All patients used the milk ladder guide (Figure 10) that represents 12 steps with examples of foods to try at home. However, we explain to caregivers that the milk ladder strategy is more a guide than a guideline, giving them freedom to progress at a pace suitable for the parent and kids, always without skipping any step.

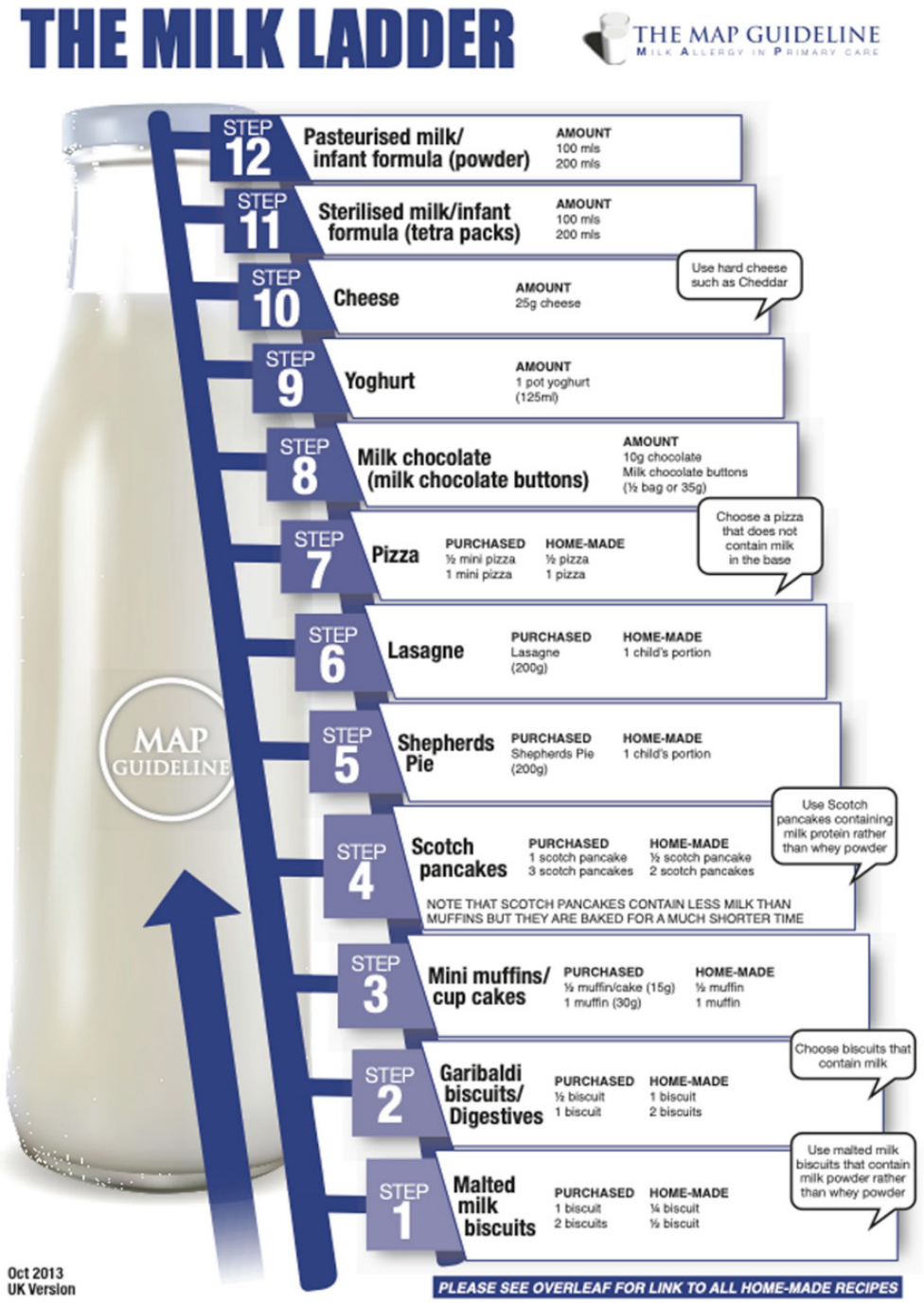


Figure 10. *Milk ladder guideline. Picture taken from Carina Venter, Trevor Brown, Neil Shah, Joanne Walsh, Adam T. Fox Clin Transl Allergy. DOI 10. 1186/2045-7022-3-23 (64)*

Pointers on using the MAP MILK LADDER for caregivers include:
Before starting the Ladder and progressing to each further Step, please ensure that your child is well at the time and that any gastrointestinal symptoms or eczema are settled.
Most children will start on Step 1. Some may already eat one or more of the foods on the Ladder. If that is the case, you need to be advised which Step of the Ladder you should start on.
The Ladder has 12 Steps, but health care professional may adjust the number of Steps to suit your child best.
The time spent on each Step will vary from one child to another (e.g., one day or 1 week) and this should also be discussed and agreed with you.
The amounts in the Ladder are given as a guidance - occasionally smaller or larger amounts may be recommended.
Each of the recipes has an egg and wheat free option (they are all soya-free) to make the Ladder suitable for those children who may have other co-existing allergies.
If the food on any Step of the Ladder is tolerated, your child should continue to consume this (as well as all the foods in the previous Steps) and then try the food suggested on the next agreed Step.
If your child does not tolerate the food in a particular Step, simply go back to the previous one (64).

3.3.2 Strict Milk avoidance

Patients with a diagnosis of IgE mediated CMPA entered immediately into strict avoidance of milk and milk products. To avoid the persistent symptoms, the elimination diet must be effective with full adherence that will include avoidance of products that could contain traces of milk or dairy. The usual strategy includes information and support from the paediatric allergist which explains the most accepted foods and nutritional substitutes. This included the use of breastmilk with or without combination with extensive hydrolysed milk or amino acid-based milk formulas. If the patient has had any direct reaction to breastmilk, avoidance of breastfeeding will be necessary also. When patients need to transition to solid food, they will have information about the proper substitute without milk or dairy. The periodic follow-up of patients was necessary to avoid prolonged use of an strict avoidance diet. Re-evaluations were usually scheduled every 6-12 months and included clinical appointments and allergy tests, such as skin prick tests and, if needed, oral food challenges, according to the allergy consultant indication. The decision of the length of avoidance of milk were decided after clinical and allergy test results. For instance, a 50% or more drop in cow's milk specific IgE in 1-2 years is a favourable prognostic indicator of natural tolerance. With this strategy the exclusion of milk products until Specific IgE values reach a desirable cut-off will be essential to decide to undergo an oral food challenge, looking for natural milk tolerance expressed in a full reintroduction of milk.

3.3.3 Early gradual reintroduction of milk

The patients were diagnosed and started with the management of early gradual reintroduction of milk as soon as they were diagnosed of CMPA at their first appointment in Hospital Miguel Servet in Zaragoza. For the first two days, they progressively increased the dose of diluted cow's milk in water (1/100 and 1/10). Then, they continued to take, at home, 2 daily intakes of the maximum amount tolerated in the hospital, requiring a rest period 2 hours after ingestion. Every week, they came to the day-ward to increase the amount (see Table 8), remaining under observation for 1 hour. All these aspects needed the collaboration of the child and their family and health personnel to achieve treatment success. It is important that throughout the process the patient and the family felt safe, and, for this reason, they received a telephone contact number which they could call 24 hours, in case the child presents with any reaction at home. In addition, parents were instructed about the identification and medical management of anaphylaxis. It is considered a success of the treatment if the patient takes an accumulative dose of 150-200ml of milk (4.65-6.2 gr of milk protein) per day. Safety measures were taken for appropriate management of this strategy, such as the administration of intakes in the day-ward and the continuous presence of a member of staff throughout the interval of time each dose increment was taken.

If the introduction was successful, a follow-up appointment should be scheduled 3-6 months after the complete introduction of milk before discharging.

Table 8. *Early gradual introduction of milk by week*

Quantity (ml/protein grams)	Date	Quantity	Date
0,5 ml (0.015 gr)		25 ml (0.77 gr)	
1 ml (0.03 gr)		35 ml (1.08 gr)	
2 ml (0.06 gr)		50 ml (1.55 gr)	
4 ml (0.12 gr)		75 ml (2.32 gr)	
7 ml (0.21 gr)		100 ml (3.1 gr)	
11 ml (0.34 gr)		125 ml (3.87 gr)	
15 ml (0.46gr)		150 ml (4.65 gr)	
20 ml (0.62 gr)		200 ml (6.2 gr)	

3.4 Patient selection and data management

3.4.1 Inclusion Criteria

Patients were included in the study if they fulfilled all of the following:

- Paediatric patients diagnosed with IgE-mediated CMPA. It was considered as diagnostic criteria:
    - The presence of cutaneous, gastrointestinal, respiratory, and/or systemic (anaphylaxis) symptoms consistent with IgE-mediated CMPA after taking infant formula and one of the following:
        - Skin Prick Test for cow's milk ≥ 3mm
        - Specific IgE levels for cow's milk >0.35 kU/L

3.4.2 Exclusion Criteria

- Patients were excluded from the study if there was insufficient documentation in their charts to complete all the relevant data, that includes for example: Type of Milk allergy (IgE or Non IgE mediated), progression of the management, laboratory findings or follow ups.

3.5 Development of the research study

Potential subjects were selected from the population who have been treated for IgE-mediated CMPA across the participant centres between 2011 and 2020 and who met all the inclusion criteria and none of the exclusion criteria.

A convenience sample of 200 patients by centre were collected from the files of the allergy clinics and recollected information through Microsoft Access® 365 version 2005 in every centre. Recollected data included: Clinic attendances, demographics, family and personal relevant past medical history, immunological values, clinical information from the first clinical contact and each subsequent clinic attendance, success of reintroduction, level of reintroduction of milk at the end of the treatment. We also obtained history of allergy symptoms experienced by the child in the intervening time. In Annex 1, the data collection form employed is attached.

3.6 Compliance/ Governance and Ethical issues

All data collection and storage complied with General Data Protection Regulation (Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons about the processing of personal data and on the free movement of such data and repealing Directive 95/46/EC (General Data Protection Regulation)). General Data Protection Regulation (GDPR) was followed by all researchers and personnel involved with this study and all researchers will have completed up-to-date training in this area.

Data coming from Spain was encrypted and transferred through a secure transfer method. Data transferred by centres was fully anonymised with no identifiers, data sharing agreement was implemented to cover this anonymised data transfer.

Only members of the clinical research team had access to the complete dataset. The analysis reports and results did not contain any personal identifiable data that would allow identification of individual participants in this study.

Approval from clinical research ethics committee (CREC) was obtained in Ireland and Spain before the recollection of data. The approval letters are shown in Annex 2.

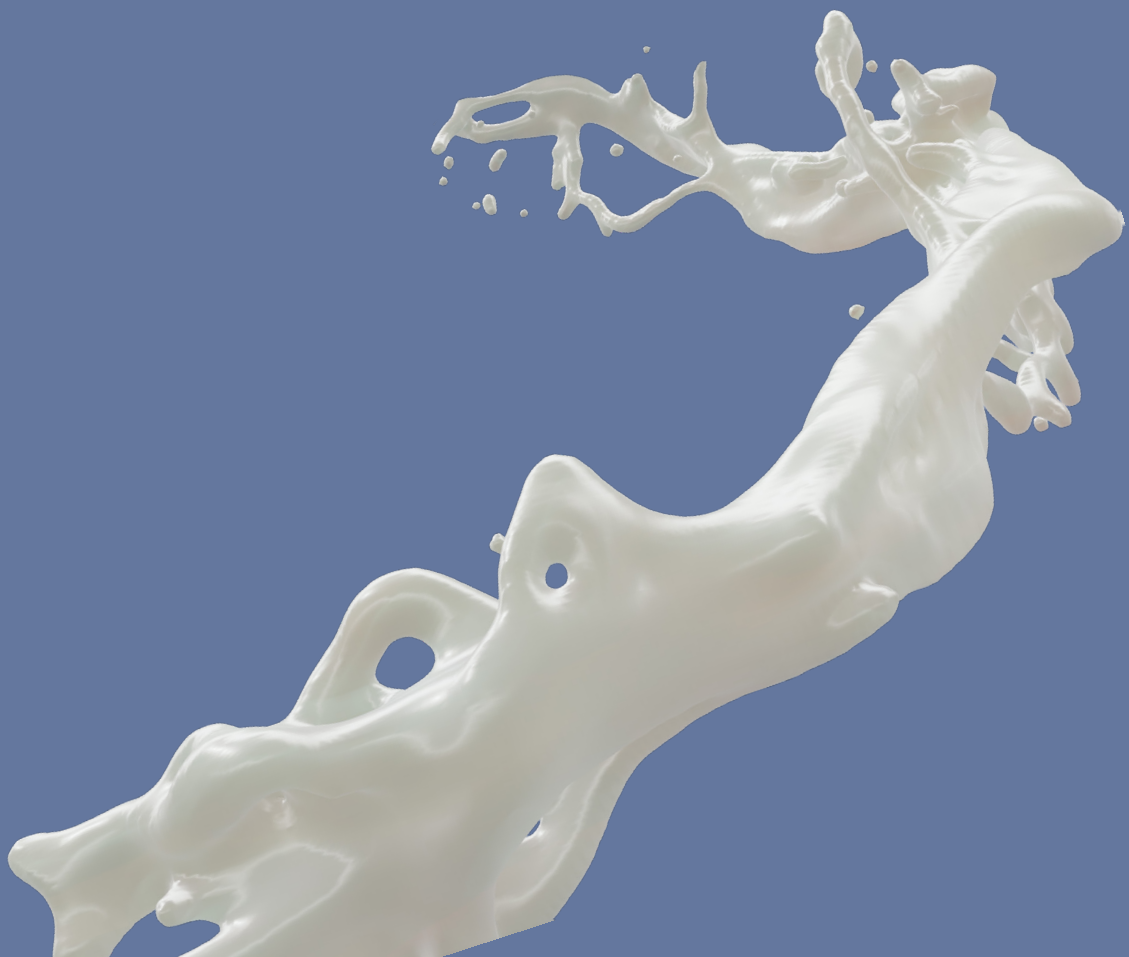
3.7 Statistical methods

For the main objective, the rate of successful treatments was compared between the three treatments, using the $\chi 2$ test. To analyze any possible confounding factors, we conducted a multivariate analysis using the success of the treatment as the dependent variable and including all the variables that showed statistical significance on univariate analysis. Confidence intervals of proportions were built by the Wilson interval method.

For the secondary objectives, a descriptive statistical analysis was carried out for all collected variables. Continuous data were expressed as mean and standard deviation (SD), whereas categorical variables were reported as percentages. Normal distribution of data was evaluated using the Shapiro-Wilk test. For non-normally distributed data, comparison was performed by employing the Mann–Whitney U test if it were two groups, or the nonparametric equality-of-medians test when it was more than two groups. The comparison of normally distributed data was performed using the t test for independent samples or the ANOVA test, depending on whether there were two or more groups, respectively. For categorical data, the $\chi 2$ test was used. Parameters displaying $p<0.05$ were considered statistically significant.

Data were analysed with Stata version 14 (Stata Corp, College Station, Texas).

4. RESULTS



4 Results

4.1 General Data

In each group, 200 patients were collected. Of these, 29 patients in the milk ladder cohort were excluded, 22 for not yet completing the milk ladder and 7 for missing information. Both strict avoidance and early gradual milk introduction obtained 200 patients each as explained in Figure 11.

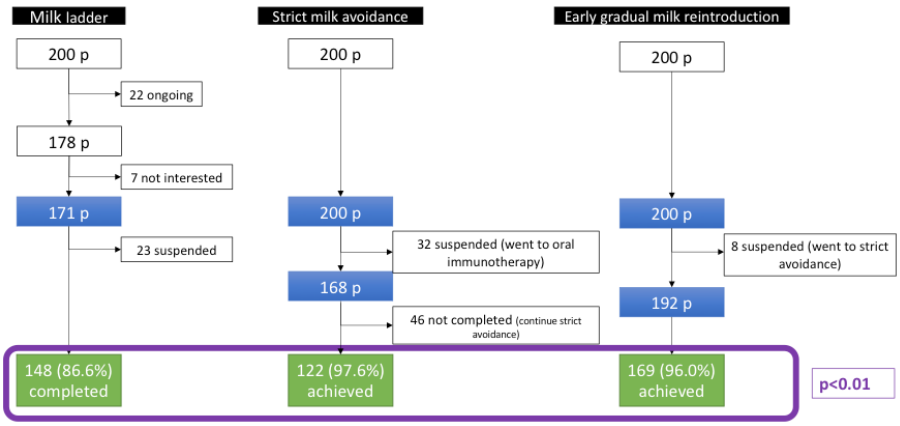


Figure 11. *Recollection of patients with different management strategies*

4.2 Demographic and baseline characteristics

The comparison between the baseline characteristics of each population is shown in Table 9. The main differences between groups were the age at diagnosis of the patients, with patients in the milk ladder cohort being diagnosed at an older age. Also, the proportion of breast-fed children was significantly lower in the milk ladder group than the remain two groups, although those who were breastfed were breastfed for a longer period of time. Lastly, there were differences between groups in the concomitant diagnosis of other allergic processes, such as rhinitis, atopic dermatitis, and other food allergies.

Table 9 *Demographic and baseline characteristics*

	Milk Ladder (n=171)	Milk Avoidance (n=200)	Early gradual milk introduction (n=200)	p
Sex male	99 (57.9%)	126 (63.0%)	96 (48.5%)	0.01
Age in Months *median (IQR)*	12 (8-26)	5 (4-7)	5 (4-7)	<0.01
Prematurity	3 (1.8%)	4 (2.0%)	8 (4.0%)	0.31
Delivery				
Vaginal	108 (63.2%)	163 (81.5%)	160 (80.0%)	
Planned c-section	25 (14.6%)	14 (7.0%)	15 (7.6%)	0.02
Emergency c-section	12 (7.0%)	22 (11.0%)	24 (12.2%)	
Unknown	26 (15.2%)	1 (0.5%)	1 (0.5%)	
Feeding before diagnosis				
Breast fed	68 (39.8%)	181 (90.5%)	179 (89.5%)	
Bottle fed	20 (11.7%)	10 (5.0%)	3 (1.5%)	<0.01
Mixed	77 (45.0%)	9 (4.5%)	14 (7.0%)	
Unknown	6 (3.5%)	0 (0%)	4 (2.0%)	
Duration of breastfeeding *(if breastfed)*	8 (6 – 12)	5 (4 – 6)	5 (4 – 6)	<0.01
Any family backgrounds	115 (67.3%)	132 (66.0%)	134 (67.0%)	<0.01
Food Allergy	57 (33.3%)	36 (18.0%)	49 (24.5%)	<0.01
Atopy	58 (33.9%)	32 (16.0%)	29 (14.5%)	<0.01
Asthma	55 (32.2%)	52 (26.0%)	32 (16.0%)	<0.01
Rhinitis	40 (23.4%)	82 (41.0%)	100 (50.0%)	<0.01
Atopic dermatitis	140 (81.9%)	122 (61.0%)	74 (37.0%)	<0.01
Asthma/viral induced wheeze	40 (23.4%)	95 (47.5%)	17 (8.5%)	<0.01
Allergic rhinitis	20 (11.7%)	39 (19.5%)	6 (3.0%)	<0.01
Any food allergy	118 (69.0%)	79 (39.5%)	66 (33.0%)	<0.01
Egg	105 (61.4%)	63 (31.5%)	61 (31.0%)	<0.01
Peanut	40 (23.4%)	13 (6.5%)	4 (2.0%)	<0.01
Other nut	13 (7.6%)	13 (6.5%)	4 (2.0%)	0.03
Other food	32 (18.7%)	26 (13.0%)	6 (3.0%)	<0.01
Carry auto-injectors	34 (19.9%)	71 (35.5%)	1 (0.5%)	<0.01

4.3 Successful reintroduction and safety

A successful reintroduction of milk treatment was achieved in 462 (80.9%) of the patients of the whole sample. The proportion of success in the milk ladder group was 86.6% (95% confidence interval (CI): 80.6 – 90.9), lower than the obtained in the early gradual milk introduction group (96.0%; 95%CI: 92.3 – 98.0). Both strategies had a significantly higher success rate than milk avoidance (61.0%; 95% CI: 54.1 – 67.5).

The duration of the treatment (Figure 12) and the number of appointments (Figure 13) were different in each strategy.

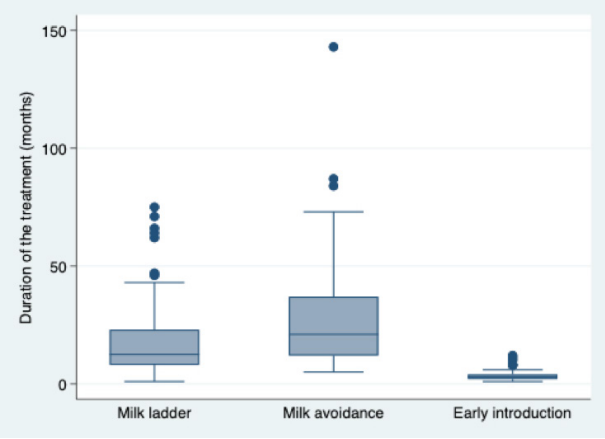


Figure 12. *Duration in months of the treatment in each strategy*

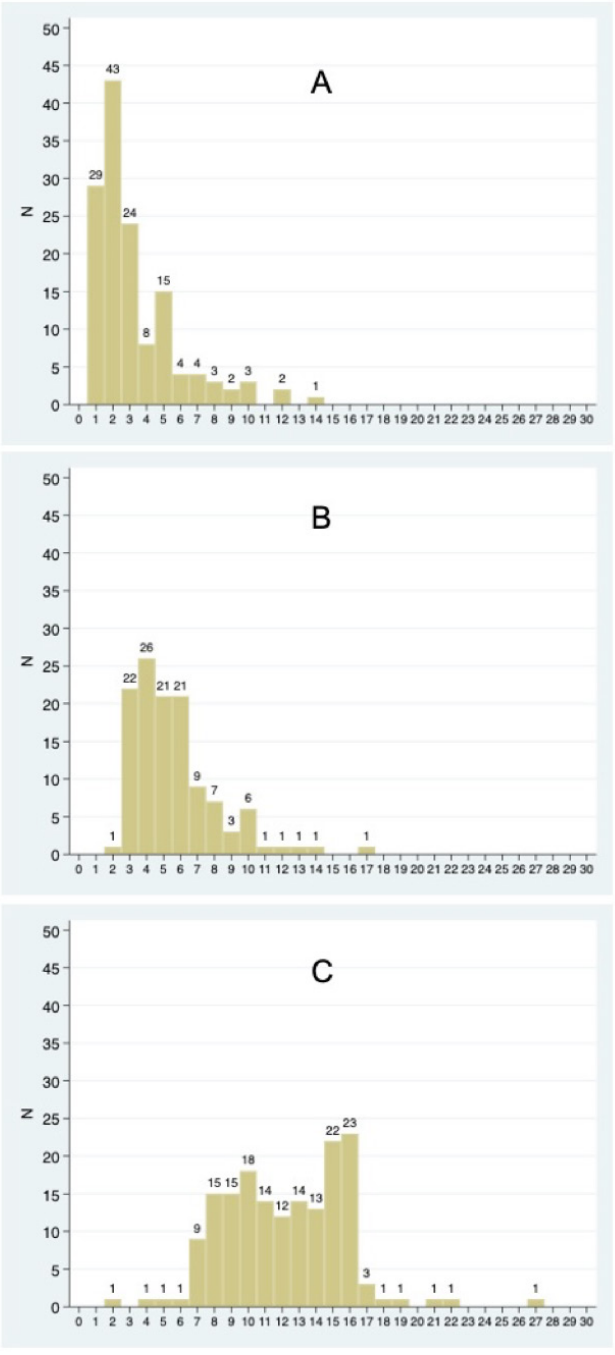


Figure 13. *Number of appointments during the treatment in each strategy (A: milk ladder; B: Milk avoidance; C: early gradual introduction)*

Milk ladder treatment had the least number of visits, while early gradual introduction of milk had an increased number of appointments in comparison to the other strategies. Regarding the duration of the treatment, early gradual reintroduction had the least duration, whereas milk avoidance was the longest treatment.

In terms of safety, there were significant differences between the three cohorts in comparison with accidental exposure to milk. Milk avoidance was the least safe treatment, with 106 patients with an accidental exposure to milk, 34 of them resulting in an anaphylactic reaction. Although the milk ladder had more patients with an exposure than early introduction (18.7% *vs.* 0.5%; p<0.01), there were no differences in the proportion of anaphylactic episodes. Table 10 show shows overall treatment features of the three cohorts.

Table 10. *Treatment features of the three cohorts. (* Significant differences only between milk avoidance and the other two strategies).*

	Milk Ladder (n=171)	Milk Avoidance (n=200)	Early gradual milk introduction (n=200)	p
Satisfactory milk Reintroduction (success)	148 (86.6%; CI95% 80.6 – 90.9)	122 (61.0%; CI95% 54.1 – 67.5)	192 (96.0%; CI95% 92.3 – 98.0)	<0.01
Duration in months median (IQR)	12.5 (8-23)	21 (12 – 37)	3 (2 – 4)	<0.01
Number of appointments median (IQR)	2 (2 – 4)	5 (4 – 6)	12 (9 – 15)	<0.01
Accidental exposure to milk	32 (18.7%; CI95% 13.6 – 25.2)	106 (53.0%; CI95% 46.1 – 59.8)	1 (0.5%; CI95% 0.1 – 2.8)	<0.01
Anaphylaxis during treatment	3 (1.8%; CI95% 0.6 – 5.0)	34 (17.0%; CI95% 12.4 – 22.8)	0 (0%; CI95% 0 – 1.9)	<0.01*

4.4 Relationship between the immunological parameters and the success of the treatment

4.4.1 Skin Prick test

A whole milk Skin prick test (SPT) was the most common procedure in the three cohorts. A SPT was done in 166 (97.1%) of milk ladder patients, 193 (96.5%) of milk avoidance patients and the 200 (100%) early introduction patients. The rate of positive results varied between groups, with 95.9% in the milk ladder group, 52.6% in the milk avoidance and 68.5% in the early introduction cohort ($p<0.01$). The Figure 14 shows the results of the SPT. In the milk avoidance group, a higher value of the SPT did associate with a failure in the treatment. That difference wasn't found in the other two groups.

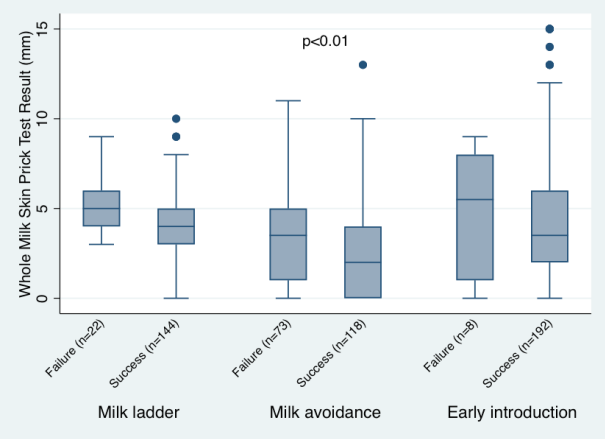


Figure 14: *Skin Prick Test results depending on the success of the treatment*

4.4.2 Specific IgE

Specific IgE component were not performed as a routine test in the milk ladder cohort. Furthermore, values for whole milk specific IgE were not obtained in 189 (94.5%) patients in the milk avoidance group, as that centre routinely use only specific IgE components (alpha lactalbumin, beta lactoglobulin, casein).

In Figure 15 the specific IgE values of whole milk (A) and components (B) are shown. In the milk ladder group, a higher value of whole milk specific IgE was associated with a failure of the treatments. The same happened in the milk avoidance group with the alpha lactalbumin, beta lactoglobulin and casein values. In the early introduction group, there was no association neither with the whole milk values nor with the components.

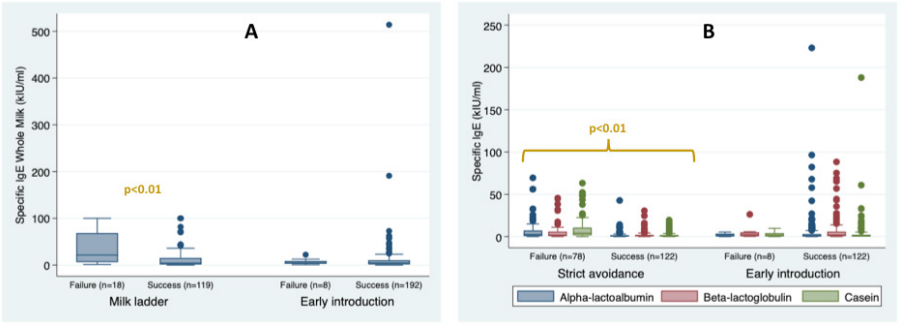


Figure 15: *Specific IgE values depending on the success of the treatment*

4.5 Association with relevant medical history of allergy

A medical history of food allergy (including egg, peanut other nuts, or other foods) and other relevant allergies (atopic dermatitis, asthma/viral induce wheeze and allergic rhinitis) was recollected. Figure 16 shows the number of patients with a relevant allergy medical history by milk strategy.

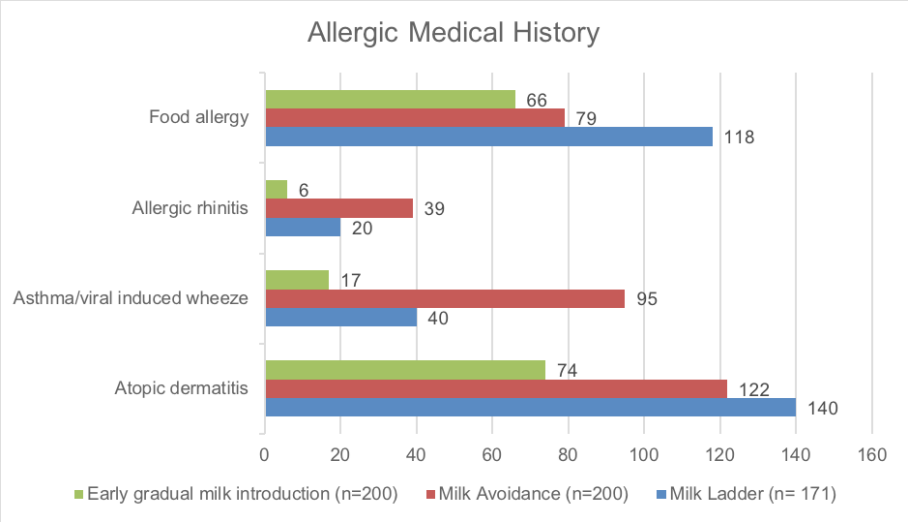


Figure 16: *Allergy medical history in each cohort*

4.6 Association between other food allergies and success in the treatment

Patients without other food allergies were more likely to succeed in their treatment. When comparing the prevalence of other food allergies and the response to treatment of each strategy, we observed that this relation was found mainly in the milk avoidance cohort. When only the milk ladder and early introduction groups were analysed, there were no differences in the rate of success. Table 11 shows the complete analysis.

Table 11. *Association between other food allergies and success in the treatment*

			Success	p
Any food allergy	Milk Ladder			
		No	42/53 (79.3%)	0.06
		Yes	108/118 (89.8%)	
	Milk Avoidance			
		No	88/121 (72.7%)	<0.01
		Yes	34/79 (43.0%)	
	Early gradual milk introduction			0.63
		No	128/134 (95.5%)	
		Yes	64/66 (97.0%)	
	WHOLE SAMPLE			
		No	**258/308 (83.8%)**	**0.06**
		Yes	**204/263 (77.6%)**	
Egg allergy	Milk Ladder			
		No	54/66 (81.8%)	0.15
		Yes	94/105 (89.5%)	
	Milk Avoidance			
		No	93/137 (67.9%)	<0.01
		Yes	29/63 (46.0%)	
	Early gradual milk introduction			0.71
		No	132/138 (95.7%)	
		Yes	60/62 (96.8%)	
	WHOLE SAMPLE			
		No	**279/341 (81.8%)**	**0.50**
		Yes	**183/230 (79.6%)**	

			Success	p
Peanut allergy	Milk Ladder			
		No	112/131 (85.5%)	0.47
		Yes	36/40 (90.0%)	
	Milk Avoidance			
		No	119/187 (63.6%)	<0.01
		Yes	3/13 (23.1%)	
	Early gradual milk introduction			0.68
		No	188/196 (95.9%)	
		Yes	4/4 (100%)	
	WHOLE SAMPLE			
		No	**397/514 (77.2%)**	**0.27**
		Yes	**43/57 (75.4%)**	
Other nut allergy	Milk Ladder			
		No	138/158 (87.3%)	0.29
		Yes	10/13 (76.9%)	
	Milk Avoidance			
		No	120/187 (64.2%)	<0.01
		Yes	2/13 (15.4%)	
	Early gradual milk introduction			0.68
		No	188/196 (95.9%)	
		Yes	4/4 (100%)	
	WHOLE SAMPLE			
		No	**446/541 (82.4%)**	**<0.01**
		Yes	**16/30 (53.3%)**	
Other food allergy	Milk Ladder			
		No	121/139 (87.1%)	0.69
		Yes	27/32 (84.4%)	
	Milk Avoidance			
		No	114/174 (65.5%)	<0.01
		Yes	8/26 (30.8%)	
	Early gradual milk introduction			0.61
		No	186/194 (95.9%)	
		Yes	6/6 (100%)	
	WHOLE SAMPLE			
		No	**421/507 (83.0%)**	**<0.01**
		Yes	**41/64 (64.1%)**	

4.7 Association between other allergies and success in the treatment

When other allergic symptomatology was analysed, we found similar results to that of the food allergies. Although the concomitant diagnosis of asthma or viral induced wheeze and allergic rhinitis was associated with treatment failure overall in the sample, in a stratified analysis we can see that this association was only found in the milk avoidance group, while in the milk ladder and early introduction groups no relationship was found between the other allergic pathologies and treatment response. Table 12 shows the complete analysis.

Table 12. *Association between other food allergies and success in the treatment*

			Success	p
Atopic dermatitis	Milk Ladder			
		No	26/31 (83.9%)	0.63
		Yes	122/140 (87.1%)	
	Milk Avoidance			
		No	53/78 (68.0%)	0.11
		Yes	69/122 (56.6%)	
	Early gradual milk introduction			0.44
		No	122/126 (91.7%)	
		Yes	70/74 (94.6%)	
	WHOLE SAMPLE			
		No	**201/235 (85.5%)**	**0.02**
		Yes	**261/336 (77.7%)**	
Asthma viral induced wheeze	Milk Ladder			
		No	115/131 (87.8%)	0.39
		Yes	33/40 (82.5%)	
	Milk Avoidance			
		No	80/105 (76.2%)	<0.01
		Yes	42/95 (44.2%)	
	Early gradual milk introduction			0.68
		No	176/183 (96.2%)	
		Yes	16/17 (94.1%)	
	WHOLE SAMPLE			
		No	**371/419 (88.5%)**	**<0.01**
		Yes	**91/152 (59.9%)**	
Allergic rhinitis	Milk Ladder			
		No	133/151 (88.1%)	0.11
		Yes	15/20 (75.0%)	
	Milk Avoidance			
		No	110/161 (68.3%)	<0.01
		Yes	12/39 (30.8%)	
	Early gradual milk introduction			0.61
		No	186/194 (95.9%)	
		Yes	6/6 (100%)	
	WHOLE SAMPLE			
		No	**429/506 (84.8%)**	**<0.01**
		Yes	**33/65 (50.8%)**	

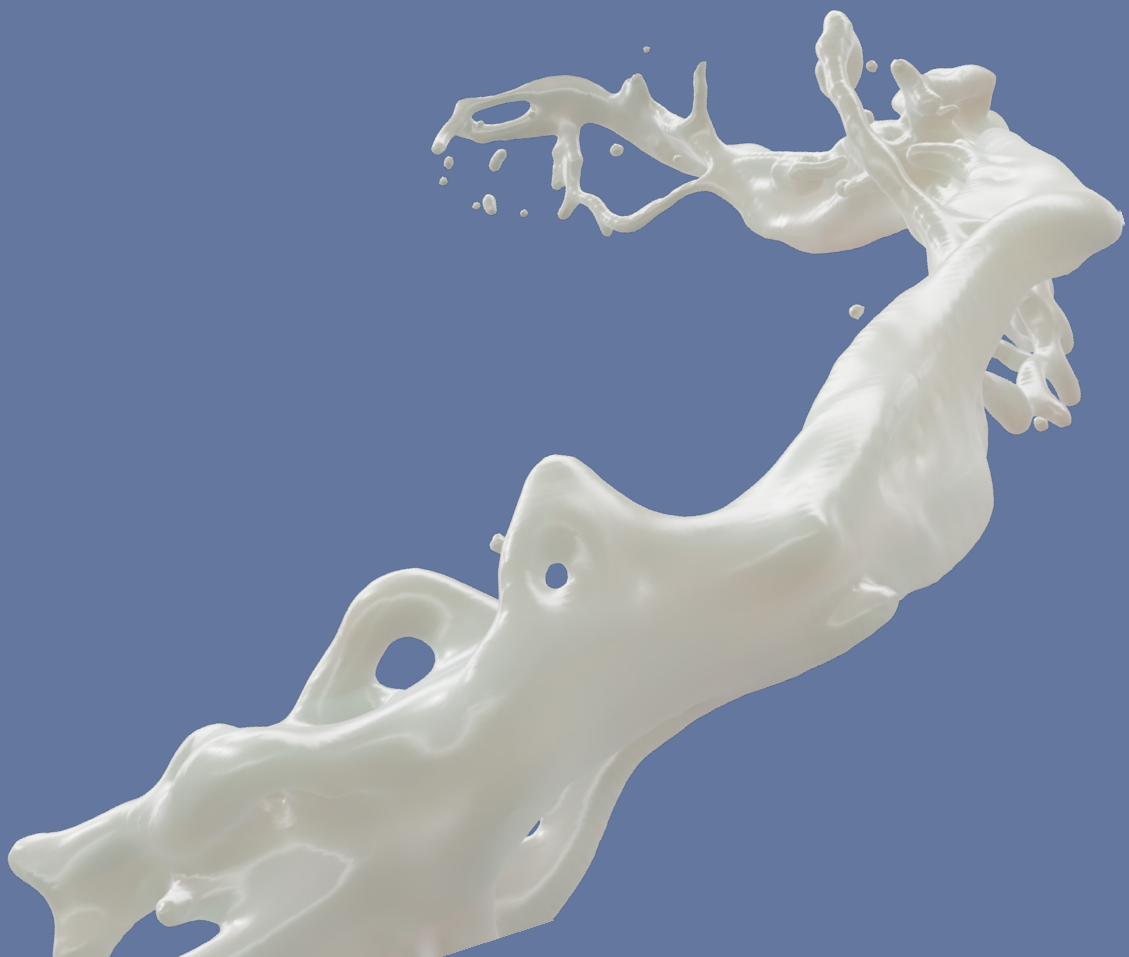
4.8 Overall analysis

A multivariate analysis comparing the three methods with potential confounders was carried out. Asthma/ viral induce wheeze, allergic rhinitis, and other nut allergy had a negative association, becoming less probable with the success of the treatment. Milk ladder and early gradual introduction were 9.24 and 4.93 times more likely to success in comparison to milk avoidance. The variables that were independently associated with the success of the treatment are described in Table 13.

Table 13. *Association between allergy diseases and acquisition of full reintroduction of milk (* compared with milk avoidance strategy)*

	OR (CI95%)
Strategy	
Milk Ladder*	4.93 (2.52 – 9.66)
Early gradual milk introduction*	9.24 (4.15 – 20.58)
Asthma/viral induced wheeze	0.39 (0.23 – 0.65)
Allergic rhinitis	0.44 (0.23 – 0.83)
Other nut Allergy	0.30 (0.12 – 0.78)

5. DISCUSSION



5 Discussion

5.1 A strong progression to an active treatment of CMPA

The involvement in the active role of management of CMPA has become more relevant in the last decades. An early introduction of food has been suggested to increase tolerance induction in allergic patients, in addition the possibility of developing sensitization via allergen exposure to the skin have been major concepts in recent years (65–67).

5.1.1 Milk avoidance

Milk avoidance is still the gold standard treatment for CMPA in many countries (15), with success rate ranging from 34% to 87% at the age of 3 (11). Avoidance is easy in theory but challenging for the family and the patient to apply. It involves extensive commitment of the dietician to provide guidance on foods to avoid which contain traces of cow's milk (70). Even though accidental exposures are common in children with food allergy, there are not so many studies that focus on this subject. Boyano-Martinez et al. recollected 88 patients with CMPA of which 40% (35 patients) had accidental exposures with only four (8%) requiring adrenaline (71). We can correlate these with our own findings, with more than 50% (106 patients) having at least one accidental exposure during their treatment, 34 (17%) of them needing adrenaline due to anaphylaxis during the management period.

5.1.2 The use of Milk reintroduction Milk Ladder

In Ireland, the MAP milk ladder is the main treatment for reintroduction of milk in children with CMPA (5). The milk ladder has been shown to be a safe and effective method of introducing baked milk and thus promoting the acquisition of milk tolerance (5,31). With 86.6% patients achieving satisfactory reintroduction of milk, our group of patients had similar outcomes with the two published articles so far, however, we can't compare our results in the case of Ball et al. due to a difference in the selection criteria (mainly due to the exclusion of patients with serious reaction and a lack of information of follow up of patients after 12 months in D'Art et al. (5,31).

We consider the use of milk ladders to act as guidance for the family rather than a clinical guideline focusing on a reintroduction option of milk for patients with CMPA. As we have explained before, we are not considering this a specific OIT management as it is not our intention on this research to label this as a treatment for a specific food allergy and rather as an active home-based introduction pathway. The rate of progression through the ladder will depend on a holistic approach, with the involvement of allergists, the general practitioner, dietetics, and nurses, all of whom are adequately prepared to support caregivers at any moment of their home introduction with the milk ladder.

5.1.3 Early gradual reintroduction of milk

Of the three treatment strategies, the early reintroduction of milk is the most difficult strategy to compare, as there are few numbers of studies published using this strategy. Four such studies were found to all have success rates of more than 97% (two of them with a 100% success rate but with less than 30 patients in each cohort) and none of them reporting a severe allergic reaction during treatment (40–43). Those publications along with the present study show that the early gradual milk introduction is a novel way of introducing milk to young infants using an OIT protocol.

5.2 Achieving our primary objective

It is important to consider that the three treatment strategies explored in this study are focused only on the reintroduction of cow's milk in CMPA and is not intended to reflect an immunomodulatory treatment or desensitization of the patient.

The main objective is to compare the success rate in full reintroduction of milk of each of the three strategies was achieved as we have shown in our results. Patients with a clinical diagnosis of IgE-mediated CMPA were treated according to the usual real-life management situation depending on the localization of their allergy department: the milk ladder in Cork, milk avoidance in Mostoles and early gradual milk introduction in Zaragoza.

We showed that milk ladder and early gradual milk introduction had a significant difference compared with milk avoidance for the primary endpoint, with an absolute difference of 25.6% and 35% respectively. Therefore, we could assume the significant effectiveness of these two managements as an option for milk reintroduction compared to strict avoidance. To our knowledge, this is the only study that has conducted a comparison between these three types of managements of CMPA. However, prospective studies must be carried out to confirm the significance of our findings.

5.3 Comparing the characteristics of each management

Regarding the secondary endpoints, there was also a statistically significant difference between the 3 groups in relation to the duration of the treatment. Early gradual milk introduction is 4 times faster than the milk ladder and 7 times faster compared to milk avoidance, with an average of 3 months of duration in treatment. The number of follow up appointments over the duration of the treatment was significantly different also among the 3 groups. Using an early gradual milk reintroduction will need an average of 12 appointments in comparison to milk ladder with 2 appointments and milk avoidance with 5 appointments. In the case of early gradual milk introduction, we considered that the practicality of this strategy may be a barrier to implementing it in some clinics, with waiting lists to attend allergy specialists varying among countries and communities. It is not unusual to have year-long waiting periods for appointments and for oral food challenges, and even longer in many cases. Therefore, many children would not have the opportunity to undergo OIT in infancy due to this discrepancy between allergy services.

5.4 Safety of each protocol

One of the major concerns about active reintroduction of milk products in CMPA patients is its relative safety and the possibility of major allergic reactions (31). Among our groups, accidental exposure to milk had a statistical difference between the 3 cohorts. Early gradual introduction of milk had only one accidental exposure. This could be explained due to the use of formula-based milk as the main way to introduce dairy and increased clinical observations of the patient due to the increased number of hospital appointments, thereby increasing the compliance of the patient. The milk ladder on the other hand had 32 patients exposed accidentally. In the case of milk avoidance, the number of accidental exposures increased significantly to 106. It is unclear why there is such a big difference in the incidence of accidental exposures between the 3 treatment strategies. A plausible interpretation of this may be that due the active role of reintroduction of several baked milk products over the first steps of the milk ladder, giving the patient less opportunity of an accidental exposure to higher doses of milk products compared to those avoiding milk. In contrast, every exposure to milk in a classical approach such as avoidance is an accidental exposure. It could also be interpreted that during the recollection of data, an accidental exposure was only considered if it gave the patient any allergic reaction symptoms, it is therefore possible that the patients on the milk ladder and early gradual introduction of milk had several accidental exposures that were not collected and did not cause an allergic reaction, becoming difficult for parents to recognize when the patient had an exposure to milk products.

With regard to anaphylaxis, the incidence of cases of anaphylaxis in the milk ladder and in the early reintroduction of milk patients compared to the milk avoidance patients was significant. Milk ladder recorded only 3 episodes of anaphylaxis in 3 different patients, none of which were caused by progression through the ladder. This is comparable to previous studies of CMPA patients who also didn't record any cases of anaphylaxis during the management and progression of milk ladder (5,31) or during the treatment of early gradual milk introduction (40,41,43).

As is seen form the results, the milk ladder and early gradual milk introduction of milk have better success rates in the number of children with full milk introduction, with less serious reactions than the complete avoidance of milk. As well as taking effectiveness and safety into consideration, the preference of the treatment strategy will depend also on the cultural context and human resources available within each centre. In Ireland, several complexities of allergic disease are treated in two allergy centres (57). Due to the increasing number of patients and the long waiting lists (in 2022 routine waiting list was longer than 12 months in both centres), the care of the patient is severely jeopardized. This could be one of the reasons for the increased age of diagnosis in comparison to the other cohorts in this study. The usual waiting list in the other 2 allergy centres (Mostoles and Zaragoza) is less than 4 months approximately. This will explain the early diagnosis and the availability to have additional appointments, something that at the moment is impossible in Irish allergy centres.

5.4 Can we rely on the usual parameters to predict an outcome?

During our training as healthcare workers with an interest in allergies, we are presented with certain almost dogmatic evidence that has not been refuted with which to base our clinical practice. One such evidence is the association between higher values of immunological parameters that include the skin prick test and specific IgE to decide a threshold that could give us a prognosis of persistence in CMPA (11,15). Another piece of evidence that is taught is the connection between other allergic diseases and its correlation with a more severe CMPA patients (72). However, based on our data analysis of the effect of immunological parameters and the presence of other allergic conditions and the effectiveness of different treatment strategies, children successfully progressed through the milk ladder and were successfully gradually introduced milk, irrespective of baseline immunological parameters or history of other allergic conditions. Therefore, we recommend that these predictive parameters be further examined in future studies when evaluating the likely outcome of treatment strategies for CMPA.

5.4.1 Immunological parameter

There are 30-35 grams of protein per litre of cow's milk (CM), with at least 20 proteins being potential allergens. Whey proteins (soluble proteins) make up 20% of CM and caseins (insoluble proteins) make up 80% of CM. Most CMPA is caused by whey proteins, but the allergy can be further exacerbated by caseins. The major milk allergens are casein (alpha-s1-, alpha-s2-, beta-, and kappa-casein) and whey proteins - β-lactoglobulin (Bos d 5), and α-lactalbumin (Bos d 4). Minor allergens, which have shown to cause allergy in only a small number of patients, include immunoglobulin, bovine serum albumin, and lactoferrin. CMPA patients are usually sensitised to more than 1 allergen, with sensitivity to both whey and casein proteins (11,73). The different cut-off values of skin prick test and specific IgE for milk and its components are described in literature to diagnose using positive predictive values for CMPA and varies depend on the prevalence of each cohort (74–76). An Italian systematic review observed that

in children less than 2 years old a skin prick test (SPT) of cow's milk extract more above 6mm and a specific IgE of milk above 5 KU/L the diagnosis of CMPA was highly likely. Baseline wheal diameter of SPT could be a good parameter to differentiate which patients could outgrow the allergy. A Japanese cohort of CMPA with a confirmed CMPA with OFC and a negative OFC after complete milk avoidance with a median avoidance of 13 months between each other described a difference of less than or equal to 6mm from baseline SPT and last SPT before Oral food challenge, however their median baseline SPT of the patient that outgroew CMPA in comparison to the patients that outgroew the allergy were not significantly different (77). Our Milk avoidance group had significant baseline difference between the patients who outgroew the allergy and patients who had persistent CMPA after an oral food challenge. Due to the real-life characteristics of our study, as we have explained before, we don't use an OFC to confirm the diagnosis, there-by the number of patients with only sensitivity to milk in immunological parameter instead of a confirmed diagnosis of CMPA is unknown. Specific IgE values had the same tendency. Recollection of only molecular components of specific IgE (Alpha lactalbumin, beta Lactoglobulin and casein) were the only serum immunological parameters recollected in our milk avoidance group with significant difference between patients that outgroew CMPA or not. These results are also described in other literature with more emphasis in the use of casein as a more accurately value (78).

In contrast to our findings in our milk avoidance cohort, the milk ladder and early gradual milk introduction did not follow the same pattern, with baseline SPT and specific IgE not correlating with the treatment outcome. It could be considered that part of this is due to the increased success in outgrowing CMPA for these 2 treatments. One of the causes could be a "theoretical immunotolerance" that is involved while using both active treatments (the milk ladder and early gradual milk introduction) compared to the passive approach of milk avoidance. The best way to clearly identify if this process is involved will be with a prospective tolerance immunological parameter study. The only certainty at this point is that the usual cut-off and threshold allergic parameters are not reliable predictive parameters in both CMPA treatments, opening the door for more structured

prospective studies to understand the tendency of this parameter in active milk introduction treatments.

5.4.2 Association with other allergies

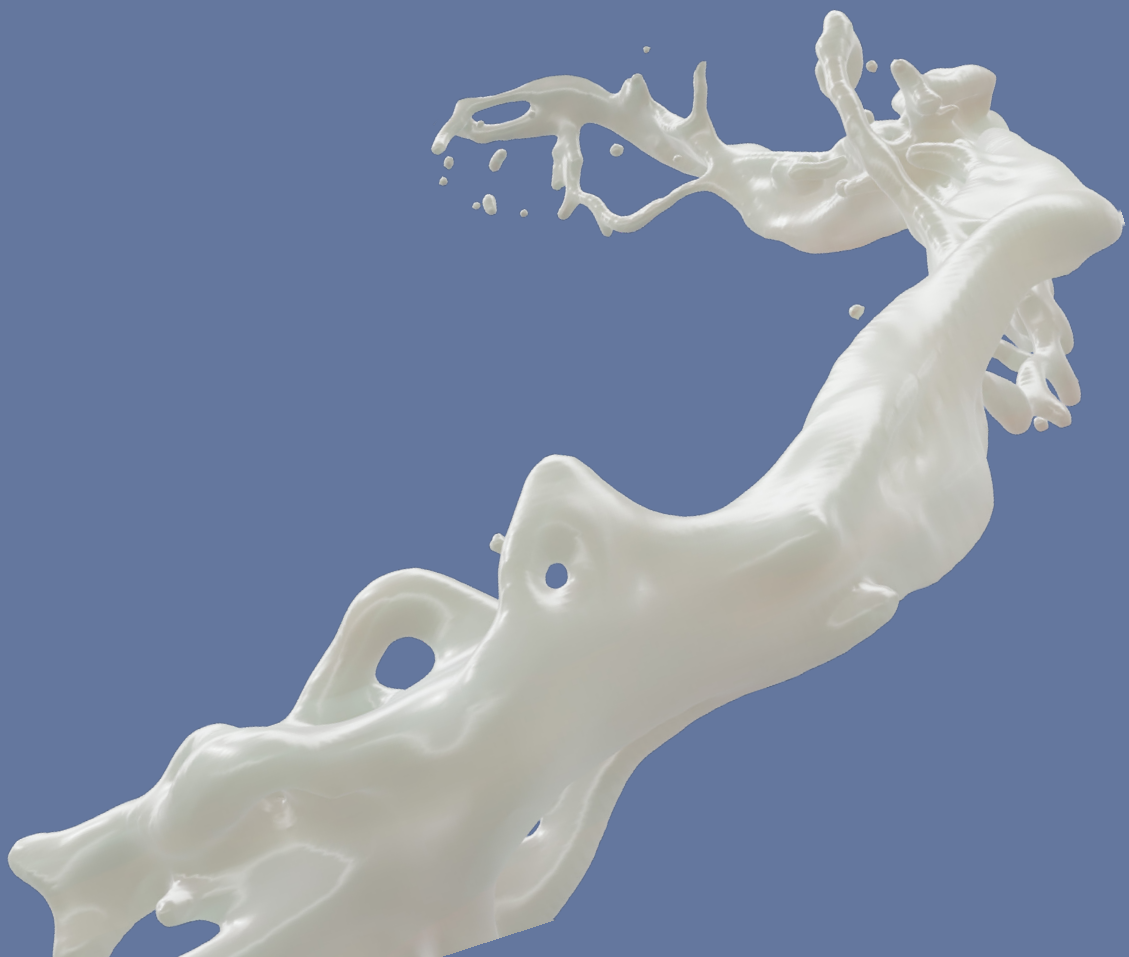
It has already been shown that the milk ladder and early gradual milk introduction behave similarly in many of the outcomes of this study. The prevalence of other food allergies and the impact of treatment outcome is no exception, with the success of the treatment being unaffected by the presence of other food allergies. Our milk avoidance patients had similar results as in other studies (72,79), revealing a steadier relationship between success of milk avoidance and a past medical history of other food allergies. The results were also similar with other types of allergies, always having a relationship with milk avoidance in all the cases.

Using these finding as shown in Table 12, there is strong evidence that the use of the milk ladder and early gradual milk introduction were 2-3 times more likely to succeed at milk reintroduction than conventional milk avoidance.

5.5 Limitations

Despite a large study population, the main limitation of this study is that it retrospectively reviews patients who underwent these treatments. Therefore, there might be missing information that was not included in the patients' charts. In order to avoid this limitation, inherent to this type of study, all the site investigators received concrete instructions about how to collect the information. That makes it unlikely that, if there was such bias, it was more present in one site than in another. In addition, we did not conduct an oral food challenge in any of the hospitals to confirm the milk allergy, as it is not the standard practice in any of the allergy centres. Further prospective studies to improve the evidence of our findings are needed in the future.

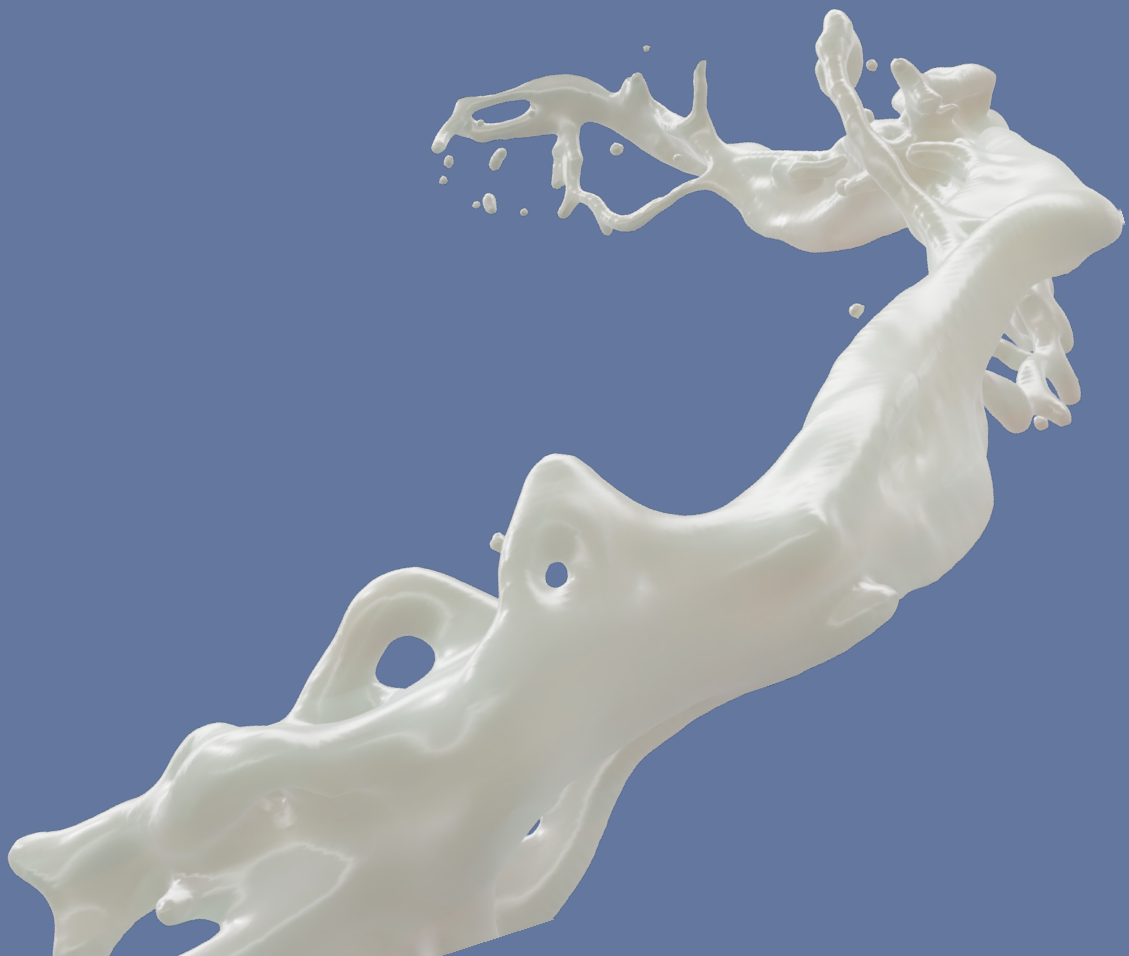
6. CONCLUSIONS



6 Conclusions

As this is the first study that retrospectively compares three real life strategies to CMPA, we can conclude that cow's milk can be successfully and safely reintroduced using different treatment strategies other than milk avoidance. All treatments show differences regarding duration, number of visits and accidental exposure to milk and therefore, we believe that these should be considered when choosing a strategy for CMPA. The results of this study will help guide the future management of CMPA and further prospective studies will need to be done to support the evidence of our findings.

7. REFERENCES



8 References

1. Han P, Gu JQ, Li LS, Wang XY, Wang HT, Wang Y, et al. The Association Between Intestinal Bacteria and Allergic Diseases—Cause or Consequence? Vol. 11, Frontiers in Cellular and Infection Microbiology. Frontiers Media S.A.; 2021.
2. Barnes PJ. Pathophysiology of allergic inflammation. Immunol Rev. 2011 Jul;242(1):31–50.
3. Wang LJ, Mu SC, Lin MI, Sung TC, Chiang BL, Lin CH. Clinical Manifestations of Pediatric Food Allergy: a Contemporary Review. Vol. 62, Clinical Reviews in Allergy and Immunology. Springer; 2022. p. 180–99.
4. Prescott SL, Pawankar R, Allen KJ, Campbell DE, Sinn JK, Fiocchi A, et al. A global survey of changing patterns of food allergy burden in children [Internet]. 2013. Available from: http://www.waojournal.org/content/6/1/21
5. d'Art YM, Forristal L, Byrne AM, Fitzsimons J, van Ree R, DunnGalvin A, et al. Single low-dose exposure to cow's milk at diagnosis accelerates cow's milk allergic infants' progress on a milk ladder programme. Allergy: European Journal of Allergy and Clinical Immunology. 2022 Sep 1;77(9):2760–9.
6. Kelleher M, Dunn-Galvin A, Hourihane JOB, Murray D, Campbell LE, McLean WHI, et al. Skin barrier dysfunction measured by transepidermal water loss at 2 days and 2 months predates and predicts atopic dermatitis at 1 year. Vol. 135, Journal of Allergy and Clinical Immunology. Mosby Inc.; 2015. p. 930-935.e1.
7. Crespo JF, Pascual C, Burks AW, Helm RM, Estebanl MM. Frequency of food allergy in a. population from Spain. Paediatric allergy and Immunology. 1995 Nov 29;(6):39–43.
8. Sekerel BE, Seyhun O. Expert panel on practice patterns in the management of cow's milk protein allergy and associated economic burden of disease on health service in Turkey. J Med Econ. 2017 Sep 2;20(9):923–30.
9. Martorell-Aragonés A, Echeverría-Zudaire L, Alonso-Lebrero E, Boné-Calvo J, Martín-Muñoz MF, Nevot-Falcó S, et al. Position document: IgE-mediated cow's milk allergy. Vol. 43, Allergologia et Immunopathologia. Elsevier Doyma; 2015. p. 507–26.
10. Lapeña Lopez de Armentia S, Hierro Delgado E. Alergia a proteínas de leche de vaca [Internet]. Madrid; 2018 Mar [cited 2022 Oct 31]. Available from: https://www.pediatriaintegral.es/publicacion-2018-03/alergia-a-proteinas-de-leche-de-vaca/

11. Giannetti A, Toschi Vespasiani G, Ricci G, Miniaci A, di Palmo E, Pession A. Cow's milk protein allergy as a model of food allergies. Vol. 13, Nutrients. MDPI; 2021.

12. Bidad K, Hossein Nicknam M, Farid R. A Review of Allergy and Allergen Specific Immunotherapy. Vol. 10, IRANIAN JOURNAL OF ALLERGY, ASTHMA AND IMMUNOLOGY. All rights reserved. Iran J Allergy Asthma Immunol. 2011.

13. Løwenstein H. Quantitative Immunoelectrophoretic Methods as a Tool for the Analysis and Isolation of Allergens (Part 1 of 3). In: Progress in Allergy Vol 25 [Internet]. Basel: KARGER; 1978. p. 1–19. Available from: https://www.karger.com/Article/FullText/314432

14. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI Molecular Allergology User's Guide. Pediatric Allergy and Immunology. 2016 May 1;27:1–250.

15. Luyt D, Ball H, Makwana N, Green MR, Bravin K, Nasser SM, et al. BSACI guideline for the diagnosis and management of cow's milk allergy. Vol. 44, Clinical and Experimental Allergy. Blackwell Publishing Ltd; 2014. p. 642–72.

16. Navarrete LV, Calvo JB, María A, Martín P, Navarrete V, Calvo B. Alergia IgE mediada a proteínas de leche de vaca. Available from: www.aeped.es/protocolos/

17. Fiocchi A, Brozek J, Schünemann H, Bahna SL, Berg A von, Beyer K, et al. World allergy organization (WAO) diagnosis and rationale for action against cow's milk allergy (DRACMA) guidelines. Vol. 21, Pediatric Allergy and Immunology. 2010. p. 1–125.

18. Montijo-Barrios E, Verónica López-Ugalde M, Ramírez-Mayans J, Salomé Anaya-Flórez M, Luis Arredondo-García J, Azevedo-Tenorio I, et al. Guía latinoamericana para el diagnóstico y tratamiento de alergia a las proteínas de la leche de vaca (GL-APLV) [Internet]. Vol. 66, Rev Invest Clin Rev Invest Clin Rev Invest Clin Rev Invest Clin Rev Invest Clin. 2014. Available from: www.imbiomed.com.mx

19. Zepeda-Ortega B, Goh A, Xepapadaki P, Sprikkelman A, Nicolaou N, Hernandez REH, et al. Strategies and Future Opportunities for the Prevention, Diagnosis, and Management of Cow Milk Allergy. Vol. 12, Frontiers in Immunology. Frontiers Media S.A.; 2021.

20. Halken S. Prevention of allergic disease in childhood: clinical and epidemiological aspects of primary and secondary allergy prevention Introduction and background.

21. Sackesen C, Altintas DU, Bingol A, Bingol G, Buyuktiryaki B, Demir E, et al. Current Trends in Tolerance Induction in Cow's Milk Allergy: From Passive to Proactive Strategies. Front Pediatr. 2019 Sep 18;7.

22. Fiocchi A, Brozek J, Schünemann H, Bahna SL, Berg A von, Beyer K, et al. World allergy organization (WAO) diagnosis and rationale for action against cow's milk allergy (DRACMA) guidelines. Vol. 21, Pediatric Allergy and Immunology. 2010. p. 1–125.

23. Shek LPC, Soderstrom L, Ahlstedt S, Beyer K, Sampson HA. Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy. Journal of Allergy and Clinical Immunology. 2004 Aug;114(2):387–91.

24. Mcwilliam V. The feasibility of a "milk ladder" approach for the introduction of cow's milk to infants with mild-moderate IgE mediated cow's milk allergy AIFA Food Allergy Research Grant 2020.

25. Cisteró Bahima AM, Alonso Lebrero E, Fernández Moya L, Somoza Álvarez ML, Madrid M, Infanta Cristina Badajoz Rotante Hospital Niño Jesús Madrid H, et al. SESIÓN DE ACTUALIZACIÓN ALERGIA A ALIMENTOS EN NIÑOS. Vol. 16, Alergol Inmunol Clin. 2001.

26. Kim JS, Nowak-Wgrzyn A, Sicherer SH, Noone S, Moshier EL, Sampson HA. Dietary baked milk accelerates the resolution of cow's milk allergy in children. Journal of Allergy and Clinical Immunology. 2011;128(1):125-131.e2.

27. Nowak-Wegrzyn A, Bloom KA, Sicherer SH, Shreffler WG, Noone S, Wanich N, et al. Tolerance to extensively heated milk in children with cow's milk allergy. Journal of Allergy and Clinical Immunology. 2008;122(2).

28. Esmaeilzadeh H, Alyasin S, Haghighat M, Nabavizadeh H, Esmaeilzadeh E, Mosavat F. The effect of baked milk on accelerating unheated cow's milk tolerance: A control randomized clinical trial. Pediatric Allergy and Immunology. 2018 Nov 1;29(7):747–53.

29. Athanasopoulou P, Deligianni E, Dean T, Dewey A, Venter C. Use of baked milk challenges and milk ladders in clinical practice: a worldwide survey of healthcare professionals. Vol. 47, Clinical and Experimental Allergy. Blackwell Publishing Ltd; 2017. p. 430–4.

30. Chomyn A, Chan ES, Yeung J, vander Leek TK, Williams BA, Soller L, et al. Canadian food ladders for dietary advancement in children with IgE-mediated allergy to milk and/or egg. Allergy, Asthma & Clinical Immunology. 2021 Dec 5;17(1):83.

31. Ball HB, Luyt D. Home-based cow's milk reintroduction using a milk ladder in children less than 3 years old with IgE-mediated cow's milk allergy. Clinical and Experimental Allergy. 2019 Jun 1;49(6):911–20.

32. Turner PJ, d'Art YM, Duca B, Chastell SA, Marco-Martin G, Vera-Berrios RN, et al. Single-dose oral challenges to validate eliciting doses in children with cow's milk allergy. Pediatric Allergy and Immunology. 2021 Jul 1;32(5):1056–65.

33. Wood RA. Food allergen immunotherapy: Current status and prospects for the future. Vol. 137, Journal of Allergy and Clinical Immunology. Mosby Inc.; 2016. p. 973–82.

34. Aragonés AM. Inmunoterapia específica con alérgenos alimentarios. Available from: www.aeped.es/protocolos/

35. Mestecky J, Bienenstock J, McGhee JR, Lamm ME, Strober W, Cebra JJ, et al. Historical Aspects of Mucosal Immunology. In: Mucosal Immunology [Internet]. Elsevier; 2005. p. xxxiii–lvi. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780124915435500048

36. Keet CA, Wood RA. Emerging therapies for food allergy. Journal of Clinical Investigation. 2014 May 1;124(5):1880–6.

37. Knol EF, de Jong NW, Ulfman LH, Tiemessen MM. Management of cow's milk allergy from an immunological perspective: What are the options? Nutrients. 2019 Nov 1;11(11).

38. Pajno GB, Fernandez-Rivas M, Arasi S, Roberts G, Akdis CA, Alvaro-Lozano M, et al. EAACI Guidelines on allergen immunotherapy: IgE-mediated food allergy. Allergy: European Journal of Allergy and Clinical Immunology. 2018 Apr 1;73(4):799–815.

39. Taniuchi S, Takahashi M, Soejima K, Hatano Y, Minami H. Immunotherapy for cow's milk allergy. Hum Vaccin Immunother. 2017 Oct 3;13(10):2443–51.

40. Reche M, Valbuena T, Fiandor A, Padial A, Cañete A, Pascual C. Early Induction Of Oral Tolerance Protocol (OTI) In Children With Cow'S Milk Allergy. Journal of Allergy and Clinical Immunology. 2011 Feb;127(2):AB24–AB24.

41. 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. Annals of Allergy, Asthma and Immunology. 2013 Dec;111(6):523–8.

42. Berti I, Badina L, Cozzi G, Giangreco M, Bibalo C, Ronfani L, et al. Early oral immunotherapy in infants with cow's milk protein allergy. Vol. 30, Pediatric Allergy and Immunology. Blackwell Publishing Ltd; 2019. p. 572–4.

43. Boné Calvo J, Clavero Adell M, Guallar Abadía I, Laliena Aznar S, Sancho Rodríguez ML, Claver Monzón A, et al. As soon as possible in IgE-cow's milk allergy immunotherapy. Eur J Pediatr. 2021 Jan 1;180(1):291–4.

44. Vila L, Beyer K, Järvinen KM, Chatchatee P, Bardina L, Sampson HA. Role of conformational and linear epitopes in the achievement of tolerance in cow's milk allergy. Clinical and Experimental Allergy. 2001;31(10):1599–606.

45. Chatchatee P, Järvinen KM, Bardina L, Vila L, Beyer K, Sampson HA. Identification of IgE and IgG binding epitopes on β- and κ-casein in cow's milk allergic patients. Clinical and Experimental Allergy. 2001;31(8):1256–62.

46. Chatchatee P, Järvinen KM, Bardina L, Beyer K, Sampson HA. Identification of IgE- and IgG-binding epitopes on αs1-casein: Differences patients with persistent and transient cow's milk allergy. Journal of Allergy and Clinical Immunology. 2001;107(2):379–83.

47. Järvinen KM, Beyer K, Vila L, Chatchatee P, Busse PJ, Sampson HA. B-cell epitopes as a screening instrument for persistent cow's milk allergy. Journal of Allergy and Clinical Immunology. 2002;110(2):293–7.

48. de Boissieu D, Dupont C. Allergy to extensively hydrolyzed cow's milk proteins in infants: Safety and duration of amino acid-based formula. Journal of Pediatrics. 2002 Aug 1;141(2):271–3.

49. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro PG. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. Allergy: European Journal of Allergy and Clinical Immunology. 2004 Sep;59(9):980–7.

50. Venter C, Brown T, Shah N, Walsh J, Fox AT. Diagnosis and management of non-IgE-mediated cow's milk allergy in infancy - a UK primary care practical guide. Clin Transl Allergy. 2013 Jul 8;3(1):1–11.

51. Venter C, Brown T, Meyer R, Walsh J, Shah N, Nowak-Węgrzyn A, et al. Better recognition, diagnosis and management of non-IgE-mediated cow's milk allergy in infancy: IMAP - An international interpretation of the MAP (Milk Allergy in Primary Care) guideline. Vol. 7, Clinical and Translational Allergy. BioMed Central Ltd.; 2017.

52. Berni Canani R, di Costanzo M, Bedogni G, Amoroso A, Cosenza L, di Scala C, et al. Extensively hydrolyzed casein formula containing Lactobacillus rhamnosus GG reduces the occurrence of other allergic manifestations in children with cow's milk allergy: 3-year randomized controlled trial. Journal of Allergy and Clinical Immunology. 2017 Jun 1;139(6):1906-1913.e4.

53. Nowak-Węgrzyn A, Lawson K, Masilamani M, Kattan J, Bahnson HT, Sampson HA. Increased Tolerance to Less Extensively Heat-Denatured (Baked) Milk Products in Milk-Allergic Children. Journal of Allergy and Clinical Immunology: In Practice. 2018 Mar 1;6(2):486-495.e5.

54. Dunlop JH, Keet CA, Mudd K, Wood RA. Long-Term Follow-Up After Baked Milk Introduction. Journal of Allergy and Clinical Immunology: In Practice. 2018 Sep 1;6(5):1699–704.

55. The European Academy of Allergy and Clinical Immunology (EAACI). Allergology in Europe: the need to harmonize medical education, disciplines, and specialties. In: Advocacy manifesto Tackling the allergy crisis in Europe - concerted policy action needed. https://www.veroval.info/-/media/diagnostics/files/knowledge/eaaci_advocacy_manifesto.pdf; 2015.

56. Fyhrquist N, Werfel T, Bilò MB, Mülleneisen N, Gerth Van Wijk R. The roadmap for the Allergology specialty and allergy care in Europe and adjacent countries. An EAACI position paper. Vol. 9, Clinical and Translational Allergy. BioMed Central Ltd.; 2019.

57. Hourihane JO, Byrne Aideen. A National Model of Care for Paediatric Healthcare Services in Ireland Chapter 11: Paediatric Allergy Clinical Strategy and Programmes Division [Internet]. Dublin; 2017 [cited 2022 Oct 31]. Available from: https://www.hse.ie/eng/services/publications/clinical-strategy-and-programmes/paediatric-allergy.pdf

58. Conlon NP, Abramovitch A, Murray G, O'Hanrahan A, Wallace D, Holohan K, et al. Allergy in Irish adults: a survey of referrals and outcomes at a major centre. Ir J Med Sci. 2015 Jun 1;184(2):349–52.

59. Martin-Mateos MA. Process for European accreditation in Paediatric Allergology. Allergol Immunopathol (Madr). 2009 Sep;37(5):264–71.

60. Garcia Perez M, Amaya Pombo C, Negro Alvarez J. LA FORMACIÓN MIR EN ALERGOLOGÍA. Alergol Inmunol Clin. 2005;20:41–7.

61. Umasunthar T, Leonardi-Bee J, Hodes M, Turner PJ, Gore C, Habibi P, et al. Incidence of fatal food anaphylaxis in people with food allergy: a systematic review and meta-analysis. Clin Exp Allergy. 2013 Dec 1;43(12):1333–41.

62. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. Journal of Allergy and Clinical Immunology. 2018 Jan 1;141(1):41–58.

63. Guler N, Cokugras FC, Sapan N, Selimoglu A, Turktas I, Cokugras H, et al. Diagnosis and management of cow's milk protein allergy in Turkey: Region-specific recommendations by an expert-panel. Vol. 48, Allergologia et Immunopathologia. Elsevier Doyma; 2020. p. 202–10.

64. Venter C, Brown T, Shah N, Walsh J, Fox AT. Diagnosis and management of non-IgE-mediated cow's milk allergy in infancy - a UK primary care practical guide. Clin Transl Allergy. 2013 Jul 8;3(1):1–11.

65. Elizur A, Katz Y. Timing of allergen exposure and the development of food allergy: Treating before the horse is out of the barn. Vol. 16, Current Opinion in Allergy and Clinical Immunology. Lippincott Williams and Wilkins; 2016. p. 157–64.

66. Sackesen C, Altintas DU, Bingol A, Bingol G, Buyuktiryaki B, Demir E, et al. Current Trends in Tolerance Induction in Cow's Milk Allergy: From Passive to Proactive Strategies. Vol. 7, Frontiers in Pediatrics. Frontiers Media S.A.; 2019.

67. Kulis MD, Smeekens JM, Immormino RM, Moran TP. The airway as a route of sensitization to peanut: An update to the dual allergen exposure hypothesis. Journal of Allergy and Clinical Immunology. 2021 Sep 1;148(3):689–93.

68. Lorente Toledano F, Isidoro M, Dávila I, Laffond E, Moreno E. Prevention of allergic diseases. In: Allergologia et Immunopathologia. Elsevier Doyma; 2007. p. 151–6.

69. Brozek JL, Terracciano L, Hsu J, Kreis J, Compalati E, Santesso N, et al. Oral immunotherapy for IgE-mediated cow's milk allergy: A systematic review and meta-analysis. Clinical and Experimental Allergy. 2012 Mar;42(3):363–74.

70. de Silva D, Geromi M, Panesar SS, Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. Acute and long-term management of food allergy: Systematic review. Vol. 69, Allergy: European Journal of Allergy and Clinical Immunology. 2014. p. 159–67.

71. Boyano-Martínez T, García-Ara C, Pedrosa M, Díaz-Pena JM, Quirce S. Accidental allergic reactions in children allergic to cow's milk proteins. Journal of Allergy and Clinical Immunology. 2009 Apr;123(4):883–8.

72. Flom JD, Sicherer SH. Epidemiology of cow's milk allergy. Vol. 11, Nutrients. MDPI AG; 2019.

73. Edwards CW, Younus MA. Cow Milk Allergy. 2022.

74. Turk. Diagnostic Laboratory Findings in CMA.

75. Franco JM, Pinheiro APSG, Vieira SCF, Barreto ÍDC, Gurgel RQ, Cocco RR, et al. Accuracy of serum IgE concentrations and papule diameter in the diagnosis of cow's milk allergy. J Pediatr (Rio J). 2018 May 1;94(3):279–85.

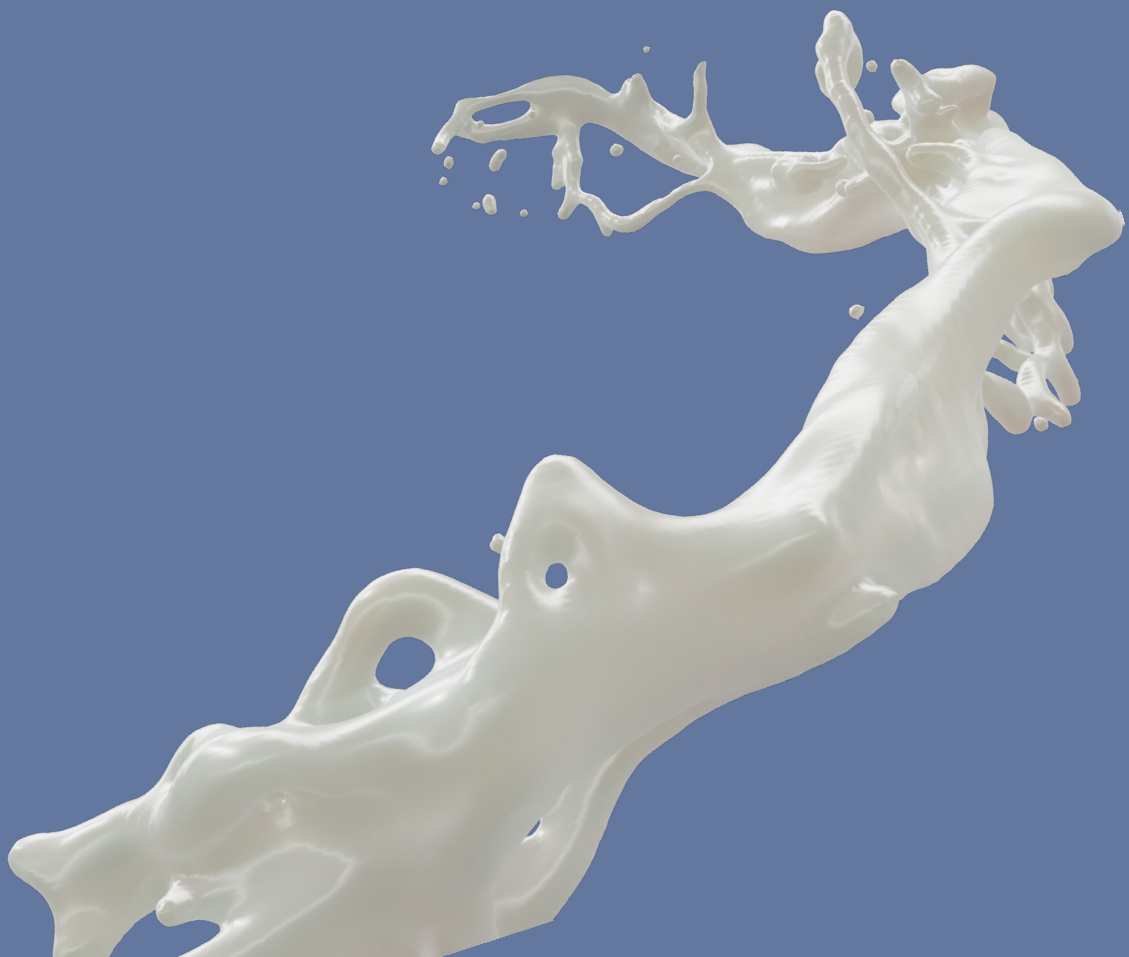
76. Cuomo B, Indirli GC, Bianchi A, Arasi S, Caimmi D, Dondi A, et al. Specific IgE and skin prick tests to diagnose allergy to fresh and baked cow's milk according to age: A systematic review. Vol. 43, Italian Journal of Pediatrics. BioMed Central Ltd.; 2017.

77. Kido J, Hirata M, Ueno H, Nishi N, Mochinaga M, Ueno Y, et al. Evaluation of the Skin-prick Test for Predicting the Outgrowth of Cow's Milk Allergy. Allergy and Rhinology. 2016;7(3).

78. Caubet JC, Nowak-W'grzyn A, Moshier E, Godbold J, Wang J, Sampson HA. Utility of casein-specific IgE levels in predicting reactivity to baked milk. Journal of Allergy and Clinical Immunology. 2013;131(1).

79. Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. Journal of Allergy and Clinical Immunology. 2007 Nov;120(5):1172–7.

9. ATTACHMENTS



9 Attachments

9.1 Glossary of Terms

BSACI	British Society of Allergy and Clinical immunology
CI	Confidence interval
CM	Cows milk
CMPA	Cows milk protein allergy
CREC	clinical research ethics committee
DRACMA	Diagnosis and Rationale for Action against Cow's Milk Allergy
EAACI	European Academy of Allergy and Clinical Immunology
ED05	Elicited dose
eHF	extensive hydrolysed formula
ETC-PA	European Training Committee – Paediatric Allergology
FA	Food allergies
GDPR	General Data Protection Regulation
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IMAP	International Milk Allergy Primary
MAP	Milk Allergy Primary
NPV	Negative predictive value
OAS	Oral allergy syndrome
OFC	Oral food challenge
OIT	oral immunotherapy
pHF	partially hydrolysed formula
PPV	Positive predictive value
SD	Standard deviation
SPT	Skin prick test
TH2	T helper 2
UEMS	European Union of Medical Specialists
WAO	World Allergy Organization

9.2 Survey of each research centre

Date recollection		
Case Number	Specific IgE Conducted	Assessment of Completion
Hospital	Total IgE	Completion Skin Prick Conducted
Treatment	Specific IgE Whole Milk (kIU/ml)	Duration Between Final Skin Prick and Completion (months)
DOB (DD/MM/YYYY)	Specific IgE Alpha-lactalbumin (kIU/ml)	Completion Skin Prick - Histamine (mm)
Gender	Specific IgE Beta-lactoglobulin (kIU/ml)	Completion Skin Prick - Whole Milk (mm)
Prematurity (<37 weeks gestation)	Specific IgE Casein (kIU/ml)	Completion Skin Prick - Alpha-lactalbumin (mm)
If YES - Gestational Age (weeks)	Specific IgE Goat's Milk (kIU/ml)	Completion Skin Prick - Beta-lactoglobulin (mm)
Delivery	Specific IgE Sheep's Milk (kIU/ml)	Completion Skin Prick - Casein (mm)
Atopic Dermatitis	Adrenaline Auto-Injector Training	Completion Skin Prick - Goat's Milk (mm)
Asthma	Carry Adrenaline Auto-Injectors	Completion Skin Prick - Sheep's Milk (mm)
Allergic Rhinitis	Number of Adrenaline Auto-Injector Carried	Completion Skin Prick - Hydrolysed Milk (mm)
Viral Induce Wheeze	Treatment Start Date (DD/MM/YYYY)	Completion Specific IgE Conducted
Other Food Allergies	Age at Start of Treatment (months)	Duration Between Final Specific IgE and Completion (months)
Food Allergy 1/2/3/4 etc	Treatment Suspended	Completion Specific IgE - Total
Specific IgE Food Allergy 1/2/3/4 etc	If NO - Reason Why Treatment Suspended	Completion Specific IgE - Whole Milk (kIU/ml)
Skin Prick Food Allergy 1/2/3/4 etc	Completed Ascent Phase (Zaragoza Only)	Completion Specific IgE - Alpha-lactalbumin (kIU/ml)
Family History - Atopy	If NO - Amount Reached Before Stopping (Zaragoza Only)	Completion Specific IgE - Beta-lactoglobulin (kIU/ml)
Family History - Food Allergy	Ascent Phase End Date (DD/MM/YYYY) (Zaragoza Only)	Completion Specific IgE - Casein (kIU/ml)

Date recollection		
Family History - Asthma	Completed Maintenance Phase (Zaragoza Only)	Completion Specific IgE - Goat's Milk (kIU/ml)
Family History - Allergic Rhinitis	If NO - Reason Why Maintenance Not Completed (Zaragoza Only)	Completion Specific IgE - Sheep's Milk (kIU/ml)
Feeding Before CMPA Diagnosis	Treatment Duration (months)	In-Clinic Reassessment
If BREASTFED - Duration (months)	Number of Follow-Up Appointments During Treatment	Duration Between Treatment Completion and Reassessment (months)
Symptoms	Total Number of Skin Pricks Conducted During Treatment	
Number of Episodes Before Diagnosis	Total Number of Blood Tests (Sp IgE) Conducted During Treatment	
Any Episode Treated as Anaphylaxis (with adrenaline)	Symptoms During Treatment (Early Intro/Milk Ladder)	
Age at Diagnosis (months)	Duration of Symptoms (months)	
Histamine (mm)	Accidental Milk Exposure	
Whole Milk (mm)	Symptoms of Accidental Milk Exposure	
Alpha-lactalbumin (mm)	Anaphylaxis During Treatment	
Beta-lactoglobulin (mm)	Number of Anaphylaxis During Treatment	
Casein (mm)	Number of Anaphylaxis Due to Accidental Milk Exposure	
Goat's Milk (mm)	Treatment End Date (DD/MM/YYY)	
Sheep's Milk (mm)	Tolerance Achieved	
Hydrolysed Milk (mm)	Age Tolerance Achieved (months)	

9.3 Ethics committee approval

Hospital Universitario de Móstoles
SaludMadrid

INFORME DEL COMITÉ ÉTICO DE INVESTIGACIÓN

Dª. Concepción Cepeda González

Presidente del Comité Ético de Investigación del Hospital Universitario de Móstoles

CERTIFICA:

Que este Comité ha evaluado el proyecto de investigación titulado:

"Comparación retrospectiva del manejo de la alergia a las proteínas de la leche de vaca mediada por IgE en tres cohortes" con nº reg. CEIC 2021/014, cuya investigadora principal es Dª. Cristina Muñoz Archidona (FE del Servicio de Pediatría y sus Áreas Específicas del Hospital Universitario de Móstoles), y considera que se cumplen los requisitos necesarios de idoneidad del proyecto en relación con los objetivos del estudio.

Este Comité aprueba que dicho estudio pueda ser realizado en este centro Hospitalario.

Lo que firmo en Móstoles a 25 de marzo de 2021.

Fdo.: Dra. Concepción Cepeda González

[redacted]

Presidente del CEI

Hospital Universitario de Móstoles
SaludMadrid

COISTE EITICE UM THAIGHDE CLINICIÚIL

Clinical Research Ethics Committee of the Cork Teaching Hospitals

Tel: +353-21-4901901
Email: crec@ucc.ie

University College Cork
Lancaster Hall
6 Little Hanover Street
Cork
Ireland

CREC Review Reference Number: ECM 4 (e) 13/4/2021

Date: 16th April 2021

Dr Juan Trujillo-Wurttele
Consultant Paediatric Allergist
Rm 2.35 Paediatric Academic Unit
Cork University Hospital
Wilton
Cork

Study Title: Comparison of IgE-Mediated Cow's Milk Protein Allergy (CMPA) Management Strategies (COWS)

Approval is granted to carry out the above study at:

Cork University Hospital, Cork, Ireland.

The following documents have been approved:

Document	Approved	Version	Date
Application Form	Yes		Signed 16/3/2021
Cover Letter	Yes		Signed 16/3/2021
Evidence of Insurance	Yes (CIS)		
CV for Chief Investigator	Yes		Signed August 2020
Data Collection Sheet	Yes		
Study Protocol	Yes	1.0	15/3/2021.

We note that the co-investigator(s) involved in this project will be:

Name	Appointment Details
Tessa Ah Heng	Final Year Medical Student UCC
Dr Roberto Velasco Zuñiga,	Consultant Paediatrician Hospital Universitario Rio Hortega (Valladolid Spain)
Dr Cristina Blasco	Consultant Paediatrician Hospital Universitario Miguel Servet Zaragoza (Spain)
Dr Cristina Muñoz	Consultant Paediatrician Hospital Universitario de Mostoles
Sarah Joy Hanley	Final Year Medical Student UCC

The date of this letter is the date of authorization of the study.

Please keep a copy of this signed approval letter in your study master file for audit purposes. The study must be carried out in accordance with General Data Protection Regulation and Health Research Regulation 2018.

You should note that ethical approval will lapse if you do not adhere to the following conditions:

1. Submission of an Annual Progress Report/Annual Renewal Survey (due annually from the date of this approval letter). **We would encourage you to keep note of this date as the CREC will not issue a reminder.**

2. Report unexpected adverse events, serious adverse events or any event that may affect ethical acceptability of the study

3. Submit any change to study documentation (minor or major) to CREC for review and approval. Amendments must be submitted on an amendment application form and revised study documents must clearly highlight the changes and contain a new version number and date. Amendments cannot be implemented without written approval from CREC.

4. Notify CREC of discontinuation of the study

5. Submit an End of Trial Declaration Form and Final Study Report/Study Synopsis when the study has been completed.

Yours sincerely

[redacted]

Professor David Kerins
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice.