

Milk Ladder for Cow's Milk Reintroduction in infants with IgE-mediated Cow's Milk Allergy: an adapted version to the Spanish population

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The prognosis of cows' milk protein allergy (CMPA) is favourable, however, CMPA may persist even into adulthood and can be severe in nature [1]. Recent anaphylaxis reports show that an increase in food-related anaphylaxis and milk has been increasingly identified as the culprit allergen in severe anaphylaxis [2-3]. This can be explained because accidental exposure to CMA, as it is something which is very ubiquitous, particularly in pre-packed foods containing milk. As a result, it is of utmost importance to develop strategies for inducing tolerance development in infants and children with CMPA.

Various observational studies have evaluated the success of food-allergy early intervention protocols [4-7] but it was not until the LEAP study [8] publication, and other well-designed randomized-trials, that we had stronger clinical evidence that the early introduction of peanut and cooked egg might prevent the development of allergy to those foods [9].

In 2011 a NICE guideline on the diagnosis and management of food allergies was published [10-11]. Based on the evidence summarized in this document, as well as, on the available data on allergenicity and the effects of food processing, an intervention to re-introduce milk and dairy into the diets of infants and children with mild to moderate non-IgE-mediated CMAP was designed [12]. The "Milk Ladder" is a plan in which the reintroduction of milk products occurs gradually and in stages. Starting with foods that contain only a small amount of well-cooked milk in a carbohydrate matrix and progressing towards un-cooked dairy products and fresh milk [10].

We have set a study to evaluate the feasibility of adapting the “Milk Ladder” intervention to children with IgE-mediated CMPA. The study is registered on [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03466931) (NCT03466931). In this article, we describe this adapted reintroduction plan and provide recipes for its appropriate implementation. Efficacy and safety data will be included in upcoming publications.

As a first step in the project, a multidisciplinary team including Allergists, Endocrinologists and Nutritionists reviewed the original protocol and recipes of the Milk Ladder. Our endocrinologist and dietician evaluated the original recipes and some of them are seldom consumed in most Spanish households so they developed recipes adequate to each step that include the same amount of milk protein of the original ones. In parallel, they looked for commercial alternatives easily available in Spanish grocery stores. The recipes that were developed are balanced, with low or no content of refined sugars, adequate fats and can be part of a healthy diet (**Table 1- supplementary material**).

The Adapted Version of the Milk Ladder (AVML) consists of the progressive introduction (4 different levels) into the participants’ diet of meals cooked with increasing amounts of cow’s milk. To ensure its correct implementation and development, seven medical visits were programmed throughout the study (**Figure 1- supplementary material**). The intervention plan is described below, as well as, in **Table 2-supplementary material**. **Table 1** describes protein content and cooking time for each recipe. Safety assessments were performed throughout the entire duration of the study. Stop criteria (box 1-supplementary material) were established to decide study termination when required. An exhaustive safety monitoring was carried out at different time points by an External Safety Committee set ad-hoc for the study.

Visit 1 (baseline assessment): Demographic data and baseline characteristics were collected. Allergy anamnesis, as well as SPT with commercial extract of cow's milk and fractions (α -lactalbumin, β -lactoglobulin and casein) were performed. Blood samples were collected for the measurement of total IgE, sIgE and IgG4 (ThermoFisher Scientific, Uppsala, Sweden), and for the determination of linear IgE and IgG4 epitopes by peptide microarray-based immunoassays [13]. Quality of life was evaluated

through the Food Allergy Quality of Life Questionnaire parent form (FAQLQ-PF) [14]. Finally, after test completion, dietary guidelines (breastfeeding/adapted hydrolysed formula and milk avoidance) and rescue medication were given to the patients' legal representatives.

Visit 2 (assessment at the start of the intervention): These evaluations were carried out 6 months after the first visit or when the patient was 12 months old. Tests and anamnesis were repeated. DBPCFC with milk was carried out and if negative, patients were considered tolerant and excluded from the study. Positive patients were considered reactive and eligible for the intervention.

Visit 3 (Level 1), Visit 4 (Level 2), Visit 5 (Level 3) and Visit 6 (Level 4): Visit 3 was held a maximum of 1 month after Visit 2. An OFC with the first food of the ladder, biscuits (equivalent milk protein content = 0.095 g/biscuit), was conducted. After 2 weeks of sustained consumption at home, patients underwent an OFC with mini-muffins (equivalent milk protein content = 0.825 g/mini-muffin). Consumption was then maintained for 3 months on a daily basis. Families were informed that milk bread rolls with the same milk protein content were commercially available, if preferred. Similarly, visit 4, visit 5 and visit 6 were scheduled to confirm the tolerance of each level of the AVML if the patient had consumed and tolerated the previous foods for 3 months. Visit 6 confirms the tolerance to 200mL of milk. If the patient tolerated it, parents were instructed to keep a daily intake of milk and milk-containing meals without any maximum amount established.

Visit 7 (final evaluation): Final visit was held 3 months (\pm 15 days) after Visit 6. The same tests and procedures as in visit 2 were followed. A DBPCFC was conducted to determine participants' tolerance level if reactions occurred at home or the patient could not progress through all the steps of the AVML. Patients were then classified as tolerant (negative results) or reactive (positive results). An early-in-life intervention such as the AVML proposed in our study, if shown to deliver effective and safe results, could modify the natural history of the disease towards an early resolution and tolerance acquisition in those who would normally outgrow on their own, or as a form of modified

OIT in those with persistent allergy. These outcomes are also expected to be associated with clear and positive benefits on the quality of life of patients and their families.

Conflict of interests

The authors declare that they have no conflicts of interest.

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FIGURE LEGENDS

Table. Cooking temperature and milk protein content for each recipe.

AVML LEVEL	Meal	Cooking temperature (°C)	Protein mg	Equivalent in milk ml
1A	Cookies	180	95	3.1
1B	Muffins	180	825	26.6
2A	Sweet pancakes	>72	260	8.4
2B	Salted pancakes	>72	1900	61.3
	Croquettes	120	1900	61.3
3A	Bechamel	120	1900	61.3
	Spanish omelette	>72	1900	61.3
3B	Bechamel	100	2600	89.3
	Banana Puree	>72	2600	89.3
	Fillings for the stew	>72	2600	89.3
	French Omelette	>72	2600	89.3
	Sweet French Omelette	>72	2600	89.3
	Spanish Omelette	>72	2600	89.3
4A	1 Yogurt (125g)	N.A.	4000	129
4B	Milk	N.A.	6200	200