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Is a slow-progression baked-milk protocol of oral immunotherapy always a safe option for children with cow's milk allergy? A randomized controlled trial

SHORT TITLE: Efficacy and safety of baked-milk oral immunotherapy

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Introduction

Immunoglobulin-E mediated-cow's milk allergy (IgE-CMA) is the one of the most frequent IgE mediated-food allergy in children in industrialized countries and may cause life-threatening anaphylaxis. The natural history of CM IgE mediated food allergy is favorable for most children. However, the clinical outcome of CMA seems worse than previously described, with less chance of recovery after the age of 3¹.

Avoidance is currently the only management approach for children with food allergies, along with educating carers about rescue treatment. However, quality of life is altered especially for children affected by ubiquitous allergens, and avoidance does completely rule out the risk of anaphylaxis due to accidental exposure².

In this context, oral immunotherapy (OIT) may be a promising alternative³. However, its main goal is to protect patients against low-threshold anaphylactic reactions, and it can cause severe adverse effects requiring the use of epinephrine³.

Using baked milk is thought to improve the safety of cow's milk OIT, as heating food is known to reduce the allergenicity of conformational epitopes⁴. Cow's milk proteins (MP)

are also altered by their interactions with other substances in the food matrix⁴. However, Nowak-Wegrzyn *et al* have shown that heated milk reactive subjects possessed IgE antibodies that still bound caseins after boiling⁴. Moreover, as highlighted by Goldberg *et al*, it remains to be determined whether baked-milk OIT can induce tolerance to raw milk⁵.

Another side of the debate is the administration schedule. Most of them consist of an initial rush up-dosing phase over 1 day in a hospital setting, followed by a maintenance phase at the same dose. However, these schedules may convey a high risk of adverse reactions due to intensive escalation.

In this context, we prospectively evaluated the efficacy and the safety of two OIT protocols in a cohort of children with persistent IgE-CMA: a cluster schedule starting immediately with raw milk (with a presumed higher risk of side effects) versus a slow-progression schedule starting with baked milk and then less and less heated milk over time (with a presumed lower risk of side effects).

Methods

Study design

Children older than 3 years¹, referred by their primary allergologist for persistent IgE-CMA, were prospectively recruited at the Department of Allergology of Armand Trousseau Children's Hospital (Paris, France) and the Department of Pediatric Pulmonology and Allergology of Lille University Hospital (Lille, France), from September 2012 to September 2015, regardless the severity of the allergic reaction (AR) and the eliciting dose at the time of initial diagnosis.

Accepted Article

Diagnosis of persistent IgE-CMA was confirmed by the occurrence of an AR during a double-blind, placebo-controlled oral food challenge (DBPCFC) performed at inclusion, associated with serum casein-specific IgE levels ≥ 0.35 kU/L. Allergic adverse reaction during DBPCFC was defined by the occurrence of urticaria and/or angioedema and/or gastrointestinal symptoms and/or respiratory symptoms, or anaphylaxis.

The DBPCFC was performed during two separate hospitalizations 8 days apart, by a trained medical and paramedical healthcare professional and according to good clinical practices.

Increasing doses were administered every 20 minutes to a cumulative dose of 5400 mg of MP (i.e. 160 mL of half-skimmed cow's milk, last single dose 2720 mg of MP). The vehicle used during the DBPCFC was potato mash⁶. The DBPCFC protocol is specified in *Supplementary Table 1*.

If an AR occurred, the challenge was immediately stopped and rescue medications were administered if necessary, according to the World Allergy Organization (WAO) recommendations.

The threshold of tolerance was defined as the last single tolerated dose, and the threshold of reactivity by the single dose triggering an objective allergic symptom. Cumulative ingested dose was also reported.

Children who did not experience any ARs at the cumulative dose of 5400 mg (last single dose 2720 mg) were considered to be naturally tolerant and were not included. Children with uncontrolled asthma, acute infection, who reacted to placebo, or those without parental consent (refusal or difficulties in understanding the protocol) were also excluded.

Included patients were discharged after the DBPCFC continuing with a daily dose equivalent to 1/10^e of the cumulative tolerated dose.

Children were randomized 1:1 to the high-risk (raw milk) or low-risk (baked milk) arm of OIT, using a random number generator. A simple and fixed randomization was chosen, with p defined as 0.5, i.e. an equiprobable allocation of compared treatment was considered.

In the low-risk arm, families were asked to increase the daily ingested dose every 15 days, at home, using baked milk through industrial preparations (*Supplementary Figure 1*). Doses were increased from 25 to 30% of MP equivalents at each step and a fix daily intake was prescribed between each step-up phase. Milk included in the preparation was gradually less and less heated over time. A switch from extensively heated baked milk to half- heated baked milk was performed once the tolerance threshold of 210 mg of MP was obtained, and from half-heated baked milk to raw milk once the tolerance threshold of 1970 mg of MP was obtained. Daily ingested dose was then increased up to a maintenance dose of 2720 mg of MP per day. Planned schedule to reach this daily intake was estimated to be about 9 months

Conversely, the high-risk arm of OIT followed a cluster build-up regimen, starting immediately with raw milk. Because this schedule is thought to have a high rate of AR³, the up-dosing was performed under medical supervision at the Department of Allergology. The interval between each up-dosing was 5 weeks, based on other centers' experiences with rush oral immunotherapy schedules for food allergens³. Each up-dosing was followed by daily home-administration of the highest tolerated dose during the build-up. Planned schedule to reach the daily intake of 2720 mg of MP was estimated to be about 5 months (*Supplementary Figure 2*).

Each family was invited to register dose-related reactions and treatments needed at home in a study notebook. The families were called systematically on a monthly basis by an experienced healthcare professional. Two epinephrine auto-injector systems were prescribed

to each patient and all families were trained about when and how to use it. The families were also educated about potential cofactors and how to avoid them or to minimize their impact, e.g. physical exercise, stress, viral infection, non-steroid anti-inflammatory drugs. Carers were told to reduce the dose by half if a child was exposed to unavoidable cofactors. Families could contact the medical team by phone in case of an incident or if they had any doubts. OIT schedule was slowed if an AR occurred and stopped if there was a need for epinephrine use, psychological aversion, poor compliance or if requested by the family.

Immunologic assessments

Serum casein-specific IgE and IgG4 levels were determined before starting the OIT and at the end of the follow-up using the ImmunoCAP system (Thermo Fischer, Uppsala, Sweden).

Serum α lactalbumin and β lactoglobulin levels were also recorded at inclusion. Casein-specific IgE/IgG4 ratio was expressed in kU/mgA.

Data collection

Parameters collected at inclusion were:

- Age, gender, age at diagnosis
- Allergic comorbidities such as food allergy or asthma,
- Threshold of tolerance, eliciting dose and severity of the AR if appropriate, and rescue medications used during the DBPCFC and the follow-up. Highly-sensitive children were defined as those with a threshold of reactivity at the time of inclusion ≤ 30 mg of MP³.

The Astier score⁷ and need for epinephrine injection were used to classify the severity of the AR. Reaction was considered severe if the Astier score ≥ 3 and/or if an epinephrine injection

was needed. Accuracy of the use of rescue medications with reported symptoms was checked by a trained medical staff.

Ethics

Both parents of each child provided written informed consent. The study was approved by the Paris Ile de France V Ethics Committee in September 2012 under the ID number 2001/42 and was posted on Clinical Trials (www.clinicaltrials.gov) under the ID number NCT02465905.

Statistics

Primary and secondary end-points

The primary end-point was efficacy, which was defined as the daily tolerated dose at home after a minimal follow-up of 5 (high-risk arm) to 9 months (low-risk arm). Patients were classified as “responders” if daily intake without symptoms was above 2720 mg of MP, as “partial responders” if between 340 mg and 2720 mg of MP and as “non-responders” if less than 340 mg of MP.

The secondary end-point was safety at home evaluated by number of reactions at home, the Astier score of these reactions and epinephrine use at home.

Sample size calculation

Given the data available in the literature⁸, the sample size was calculated based on the primary endpoint and especially full response to OIT. Assuming one third of successful OIT in the high-risk arm³ versus two thirds in the low-risk arm⁸, 23 subjects in each arm were required for a power of 80% and an α error of 0.05.

Analysis

The analysis included all children, even them that had to withdraw from the protocol. Continuous variables were expressed using median and interquartile range after testing normality using Shapiro-Wilk test. Univariate comparative analysis was first performed to assess differences in clinical and biological parameters between groups. Comparison between groups was performed for categorical variables using Chi2-test if conditions met, or by Fisher-exact test otherwise.

Wilcoxon-Mann-Whitney test was used to compare continuous variables between groups.

Kinetics of continuous variables were studied after matching using the Wilcoxon rank test.

The efficacy end-point could not be dissociated from time. Thus, a survival analysis was needed to study the event of interest “to be at least a partial responder”. We used Kaplan-Meier survival estimator and log-rank test to compare the two arms.

Tests were two-sided, with a type I error set at α of 5%. p-value was considered significant if <0.05 .

Results

50 children underwent the initial DBPCFC. Among them, nine were excluded because of a threshold of tolerance above 2720 mg of MP. Characteristics of these children did not differ from those who entered the OIT protocols, except for serum casein-specific IgE levels, IgE/IgG4 ratio and serum α -lactalbumine-specific IgE levels (*Supplementary Table 2*).

The remaining 43 patients (86%) entered the OIT program. Two were excluded a few days after the DBPCFC due to psychosocial issues. Finally, 41 children were retained for analysis (*Supplementary Figure 3*).

Characteristics of the OIT cohort at baseline (Supplementary Table 3)

Children were on average 6 years old, 61% were over 5 y and 19.5% over 10 y. 85.4% had an associated asthma and 65.9 % had at least one other food allergy.

The threshold of reactivity of the DBPCFC for these children was 68 mg of MP [17-340] and tolerated dose was 34 mg of MP [17-170]. Cumulative ingested dose eliciting the reaction was 132 mg of MP [30 – 642]. Ten children (24.4%) were classified as highly sensitive (threshold of reactivity 11.9 mg of MP [3.4- 17]). Twelve children (29.7%) experienced a severe reaction: 11 (26.9%) had an Astier score ≥ 3 and three (7.3%) required an epinephrine injection.

Eighteen patients were randomized to the high-risk arm of OIT and 23 to the low-risk arm.

In the high-risk arm, 61.1% were boys, aged 4 [3-7] at inclusion; initial diagnosis of CMA was made at 2 months old [4-5]; 72.2% had an associated other food allergy; and 83.3% associated asthma. The tolerated dose during the DBPCFC was 119 mg of milk protein [34-170]. Cumulative ingested dose eliciting the reaction was 302 mg of MP [132 – 642]. Serum casein-specific IgEs were 12.4 kU/L [6-26], IgG4 3.1 mgA/L [1.23-4.25] and IgE/IgG4 ratio 5.5 kU/mgA [3.7-6.6]. Serum α lactalbumin-specific IgEs were 7.43 kU/L [1.40-13.6] and β lactoglobulin-specific IgEs were 2.94 kU/L [1.25-9.42].

In the low-risk arm, 52.2% were boys, aged 7 [5-10] at inclusion; initial diagnosis of CMA was made at 4 months old [1-8]; 60.9% had an associated other food allergy; and 87%

associated asthma. The tolerated dose during the DBPCFC was 34 mg of milk protein [6.8-170]. Cumulative ingested dose eliciting the reaction was 132 mg of MP [30 – 302]. Serum casein-specific IgEs were 16.2 kU/L [4.2-60.9], IgG4 2.9 mgA/L [1.3-12.3] and IgE/IgG4 ratio 5.5 kU/mgA [0.8-10.5]. Serum α lactalbumin-specific IgEs were 4.17 kU/L [1.84-7.43] and β lactoglobulin-specific IgEs were 3.55 kU/L [0.93-16.4].

The clinical and biological parameters in each arm were not significantly different except for age (Table 1).

Efficacy and immunological response at the end of follow-up

None of the children were lost to follow-up. The average follow-up period was 18 months [10- 18]. Fifteen children (36.6%) were classified as responders, 11 (26.8%) were partial responders, with an average gain in threshold of tolerance of 697 mg [27.2-2550], and 15 children (36.6%) remained non-responders. No difference was found between arms according to the survival analysis ($p=0.18$).

The higher the tolerated dose was at baseline, the higher the gain in threshold of tolerance at the end of the follow-up: only 104 mg for highly-sensitive children versus 1802 mg for the others ($p=0.02$). No difference was found in the gain of tolerance threshold between the OIT arms. Average daily ingested dose of MP was 1258 mg [476-2720] in the high-risk arm *versus* 714 mg [106-2720] in the low-risk arm ($p=0.24$).

Serum casein-specific IgE levels remained similar during the follow-up, from 12.4 to 16.2 kU/L, whereas casein-specific IgG4 levels significantly increased from 2.93 [1.23-10.9] to 5.83 [1.84 – 17.35], $p<0.01$. The casein-specific IgE/IgG4 ratio also decreased during the follow-up period although the difference was not significant ($p=0.07$).

Safety of OIT

Twenty-three children (56.1%) experienced at least one AR at home. Among them, 12 (52.2%) experienced multiple reactions and 11 (i.e. 26.8 % of the entire cohort) experienced a severe AR. Abdominal pain or transient diarrhea were the most commonly reported reactions in children who experienced mild to moderate AR (n=6), without a significant difference between the arms, and require interruption of the OIT for 2 of them because leading to compliance issue (refusal from the child to take the daily dose). Among these 2 children, one was in the high-risk arm and the other in the low-risk arm.

Five children (13.5% of the entire cohort) required an epinephrine injection at home, by parents for all of them, with an average eliciting dose of 272 mg of MP [88.4 – 1190] and at various intervals since the beginning of the OIT program (occurrence after a period of 4.8 to 17 months). Strict avoidance was prescribed for two of these children. Of the remaining three, one child continued to ingest a very low dose (20 mg of MP/day), another a dose of 140 mg of MP/day, and the last child finally reached a daily dose of 2040 mg of MP.

No difference was found in the total number of ARs or Astier scores of the children with ARs ($p=0.43$ for both) between the arms. Sixteen ARs occurred in the high-risk arm, and 24 in the low-risk arm (13 with heated baked milk, 10 with half-heated milk and 1 with raw milk) ($p=0.44$). The rate of epinephrine use at home was similar in both arms of OIT, 11.8% (n=2) in the high-risk arm versus 14.3% (n=3) in the low-risk arm ($p=1$) (*Supplementary Table 4*). In the low-risk arm, epinephrine use occurred with heated baked milk for two children and with half-heated milk for one. Reactions requiring epinephrine use were not related with the switch from extensively heated baked milk to less heated baked-milk and all occurred during a maintenance phase. Timeframe figure showed that adverse reactions could occur whenever during the follow-up, although they seem slightly more frequent at the beginning of the protocol for the low-risk arm (Figure 1).

Discussion

In children with persistent IgE-CMA, an OIT approach starting with baked milk is not always better tolerated and efficient than an OIT starting with raw milk. We identified three groups of patients in the OIT group: a responder group, a partial responder group and a non responder group. The initial threshold of reactivity in the whole cohort, on average 34 mg of 286 MP, was quite low and nearly 25% of children were highly-sensitive. These highly-sensitive children had a lower gain in threshold of tolerance at the end of follow-up. Most of the children included experienced an AR during the program. In most cases the reactions were mild to moderate, but five epinephrine injections were nevertheless necessary at home.

Thus, OIT may be an at-risk procedure in children with persistent CMA even using a slow-progression schedule with baked milk.

Strengths and limits of the study

The main strength of our study is that, to our knowledge, it is the first randomized study comparing efficacy and safety of two schedules of cow's milk OIT. Family adherence to the protocol was excellent and no children were lost to follow-up, probably due to the initial therapeutic education program, an OIT notebook and regular phone calls, even if this was not formally evaluated. Inclusion of anaphylactic children also allowed us to evaluate the outcomes of OIT in this particular group of patients.

One limit could be that we did not differentiate by a food challenge children only allergic to raw milk, children only allergic to baked milk, and children allergic to both. However, the mean level of serum casein-specific IgEs was high and very similar in both arms and this may have limited this bias as a high level of casein-specific IgE is predictive of reactivity to baked

milk⁵. We also did not perform a milk challenge at the end of the follow-up so we cannot conclude on sustained unresponsiveness to milk proteins.

Children were older in the low-risk arm, despite randomization. Despite that we did not identify any mistakes in the randomization procedure, 10% of children in the low-risk arm were teenagers whereas none was in the high-risk arm. This could have influenced the safety and efficacy results according to specificities of teen-age, especially regarding exposition to potential cofactors and poorer compliance. However only one children requiring epinephrine injection at home was a teenager.

Furthermore, the relatively severe phenotype of CMA children recruited in the study does not allow us to extend our results to all children with persistent CMA.

At last, our results cannot be reduced to a simple comparison between raw and baked-milk OIT, as the design of the two schedules was different. However, although we minimized risks in the baked-milk arm by using a slow-progression schedule, we did not show a significant difference in the safety and the efficacy outcomes between the two arms.

Reasons for unsuccessful OIT and risk of adverse events

Unexpected findings were that, although non significant, rates of partly or complete response were lower in the low-risk arm and associated with a higher rate of adverse reactions in this group. Thus, AR are not uncommon even using baked milk ⁹. Indeed, baked milk was thought to be safer because of heating and the matrix effect⁵, this aspect might be overestimated. In Goldberg *et al*'s recent study, 15 children who previously failed to complete an OIT program with raw milk were enrolled in a baked-milk OIT protocol. Although OIT was conducted very slowly with baked milk, 8/11 (73%) of failures were due to AR and two children experienced anaphylactic episodes requiring epinephrine use⁵. In the cohort we present here,

prior administration of extensively heated baked milk did not prevent allergic reaction to lesser heated milk, suggesting a particular underlying phenotype.

Study of potential biomarkers to predict tolerability of baked milk, such as serum specific IgE levels and skin prick test wheal diameters, is still ongoing⁹.

Compliance is another issue that we could address in a home-based dosing schedule. Previous reports have shown that as few as 20% of patients are truly compliant to oral immunotherapy¹⁰. Compliance was not formally evaluated in our study; although families were highly motivated and sent to our center by their referent allergologist, we could not exclude a lower compliance in the low-risk arm, given to less supervised doses. According to the literature, up-dosing schedules were sometimes performed at home, sometimes at the hospital^{3,5}. According to our results, we advice that in low-reactive children, the most prudent approach would be to perform a supervised up-dosing schedule even when using baked milk.

In conclusion, risk of severe adverse events is real even in OIT programs using baked milk. Even if many patients undergo successful OIT without major safety concerns, this is not the case for all. The benefits of OIT with baked milk in accelerating recovery in children with a severe phenotype of CMA, characterized by a low threshold of reactivity at baseline, a history of anaphylaxis and associated asthma, should be carefully evaluated in specialized centers, trained to manage highly-sensitive patients. Pretreatment by omalizumab could be an option for these children.

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Conflict of Interest Statement

Authors have no conflict of interest to declare in relation with this study.

Additional Content

Additional content may be find in the supporting information online files (*Supplementary materials 1 & 2*).

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Table 1 – Clinical and biological parameters at baseline according to the oral immunotherapy arm

	“High-risk” arm* (n=18)	“Low-risk” arm* (n=23)	p-value ‡
Male sex (n,%) **	11, 61.1	12, 52.2	0.75
Age (years) ***	4 [3-7]	7 [5-10]	0.03
Age at diagnosis (months) ***	2 [4-5]	4 [1-8]	0.47
Other food allergy (n,%)**	13, 72.2	14, 60.9	0.52
Associated asthma (n,%)**	15, 83.3	20, 87	1
Tolerated dose during DBPCFC * (mg of milk proteins) ***	119 [34-170]	34 [6.8 -170]	0.15
Serum casein-specific IgE levels (kU/L) ***	12.4 [6-24]	16.2 [4.2-60.9]	0.94
Serum casein-specific IgG4 levels(mgA/L) ***	3.1[1.2-4.3]	2.9 [1.3-12.3]	0.61
Serum casein-specific IgE/IgG4 ratio (kU/mgA) ***	5.5[3.7-6.6]	3 [0.8-10.5]	0.26
Serum α lactalbumin-specific IgE levels (kU/L) ***	7.4 [1.4-1.36]	4.2[1.8-7.4]	0.69
Serum β lactoglobulin-specific IgE levels (kU/L) ***	2.9 [1.3-9.4]	3.6[0.9-16.4]	0.83

* “High-risk” arm is defined as the cluster schedule starting with raw milk, “low-risk” arm as the slow-progression schedule starting with baked milk.

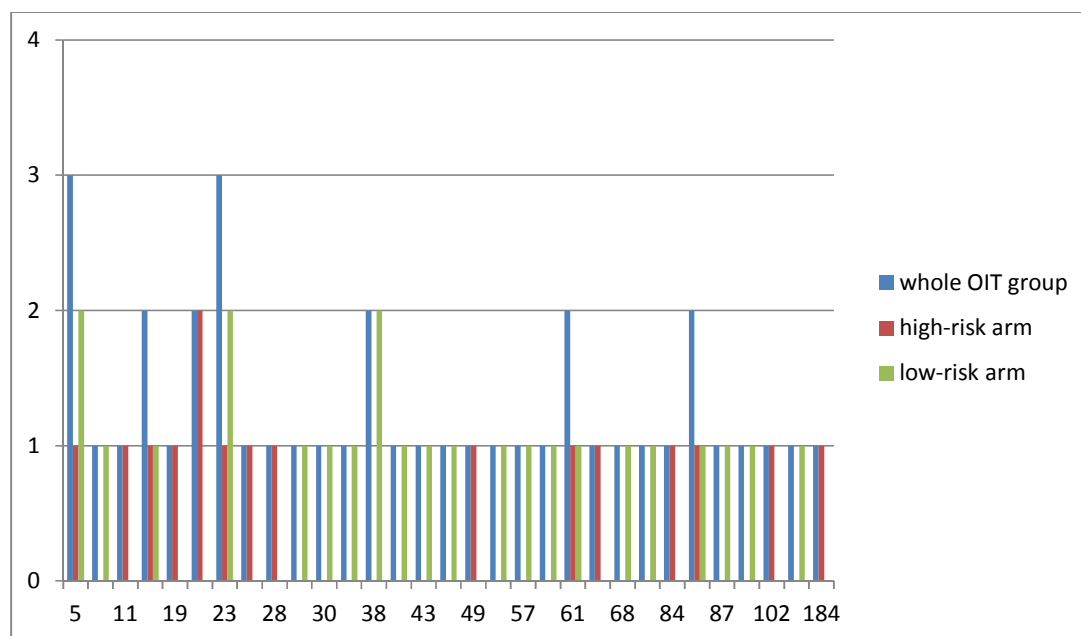
‡ DBPCFC = double blind, double-placebo food challenge

** Comparison between groups was performed for categorical variables using Chi2-test if conditions met, or by Fisher-exact test otherwise.

*** Median [interquartile range], for not normal continuous parameters. Normality was checked using Shapiro-Wilk test. Wilcoxon-Mann-Whitney test was used to compare continuous parameters between groups.

‡ p-value was considered significant if <0.05.

Figure 1. Timeframe of the occurrence of adverse reactions. Number of adverse reactions is on the vertical axis, period since inclusion (in weeks) on the horizontal axis



Supplementary Figure 1. Schedule of the low-risk arm

First part of the schedule: extensively heated milk

Minimal starting dose:

Véritable Petit Beurre®: 1/10e (0.13 mL of milk, e.g. 4 mg of milk proteins)

Increase every 15 days by approx. 25 to 30% at each step

Final dose to reach:

Prince Tout Choco®: 1 (4.4 mL of milk, e.g. 150 mg of milk proteins)

+ Véritable Petit Beurre®: 1 ½ (1.9 mL of milk, 60 mg of milk proteins)

= 6.3 mL of milk, e.g. 210 mg of milk proteins

Once the threshold of 210 mg is obtained, switch to half-heated milk



Second part of the schedule: half-heated milk

The ingested dose was reduced at the beginning of the schedule to take into account the lesser degree of heating

Starting dose:

Kinder Barre 12.5 g ®: ½ square (approx. 3.5 mL of milk, e.g. 120 mg of milk proteins)

Increase every 15 days by 25 to 30% at each step

Final dose to reach:

"Kinder Surprise" ®: 1, or Kinder Barre 12.5 g ®: 5 squares

(approx. 58 mL, e.g. 1970 mg of milk proteins)

Once the threshold of 1970 mg is obtained, switch to raw milk



Third part of the schedule: raw milk

The ingested dose was reduced at the beginning of the schedule to take into account the lesser degree of heating

Starting dose: 50 mL (approx. 1700 mg of milk proteins)

Increase every 15 days by 25 to 30% at each step

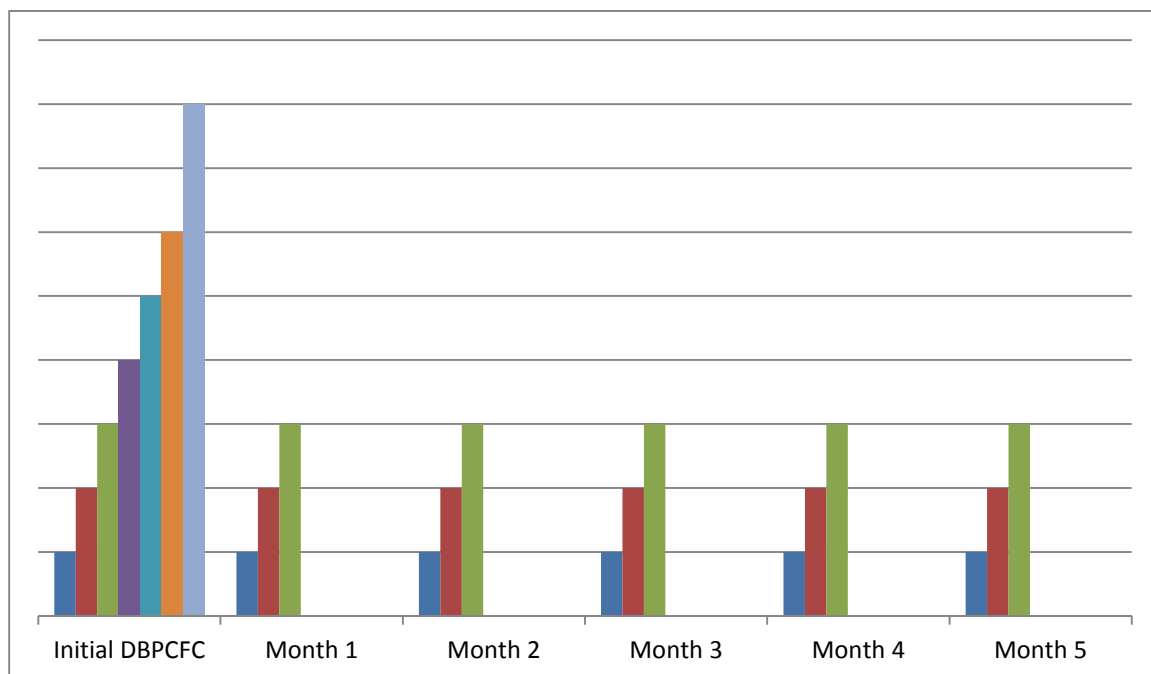
Final dose to reach:

80 mL of raw milk (approx. 2720 mg of milk proteins)

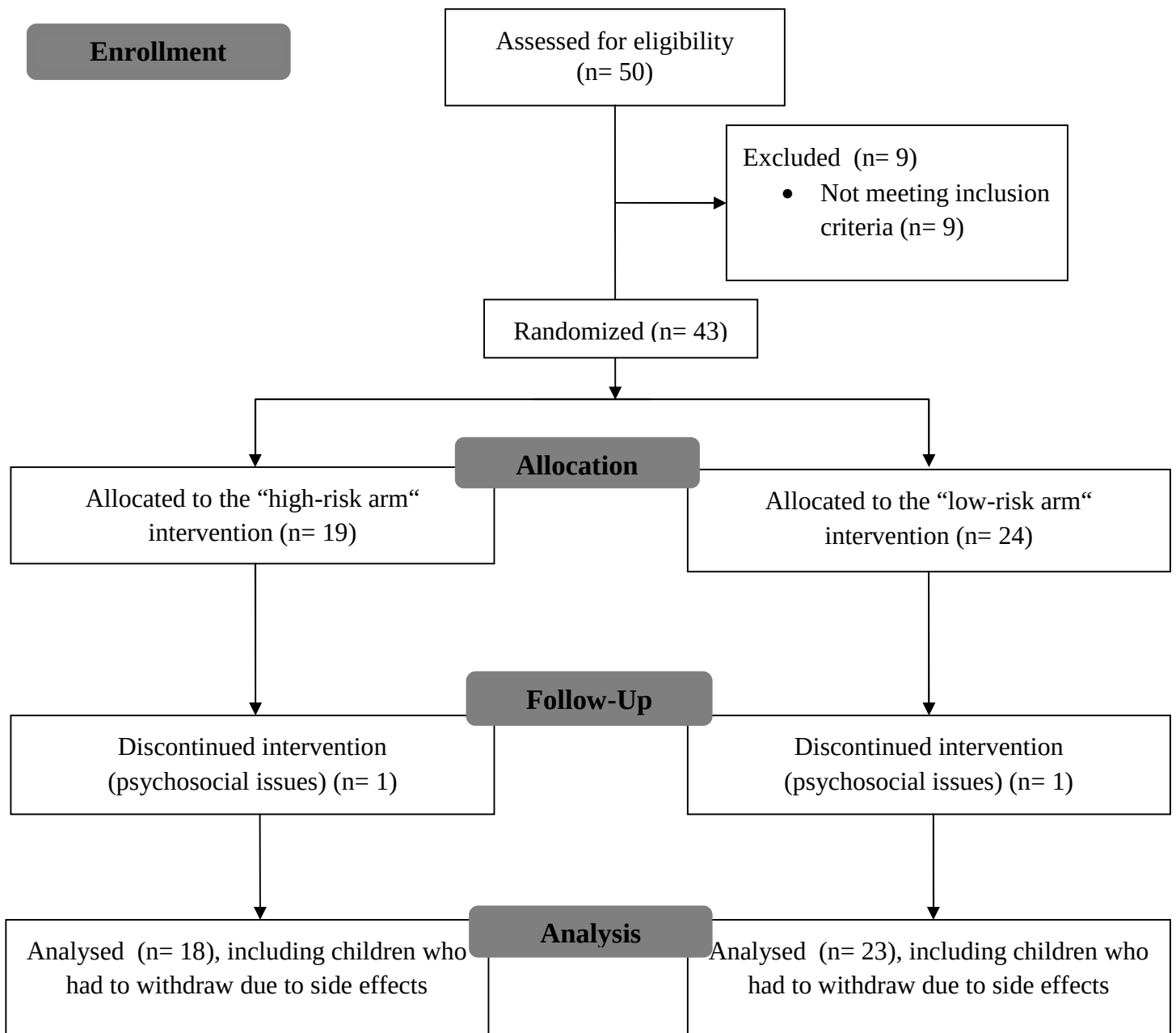
Once the threshold of 2720 mg is obtained, maintain this dose daily

Supplementary Figure 2 – Schematic schedule of the high-risk arm

Supervised up-dosing was planned each 5 weeks, and consisted in 3 increasing doses of raw milk. The child was discharged with the last tolerated dose to be taken daily at home until the next supervised up-dosing, 5 weeks later. Colors are strictly indicative.



Supplementary Figure 3. CONSORT Flow Diagram



Supplementary Table 1- Schedule of the oral food challenge. Increasing doses of 3.4% raw cow's milk (or placebo when appropriate) were administered every 20 minutes, to a maximal cumulative dose of 5400 mg of milk protein.(160 mL of raw milk). Vehicle used was potato mash.

Ratio milk/water	Volume of undiluted cow's milk (mL)	Amount of cow's milk proteins (mg)
Dilution : 1/10	0.02	0.68
-	0.05	1.7
No dilution: 1/1	0.1	3.4
	0.2	6.8
	0.5	17
	1	34
	2	68
	5	170
	10	340
	20	680
	40	1 360
	80	2 720

Supplementary Table 2 - Equivalents used from industrial preparations

Cookies are known to be baked for 12 mn at 180°C. Milk in sweets is dried at 85°C and then baked for 15 mn at 72°C.

Cookies	Volume of heated cow's milk (mL)	Amount of cow's milk proteins (mg)
Véritable Petit Beurre Lu ®	1.3	40
Petit Prince Tout Choco Lu ®	4.4	150
Sweets	Volume of half-heated cow's milk (mL)	Amount of cow's milk proteins (mg)
Kinder Barre 12.5 g ®	35	1200
Kinder Maxi ®	58	1970
Kinder Surprise ®		

Supplementary Table 3– Clinical and biological parameters of the overall population at baseline

	NT group** (n= 9)	OIT group *** (n=41)	p-value ¥
Male sex #	77.8	56.1	0.29
Age (years) ‡	5 [3-6]	6 [4-8]	0.40
Age at diagnosis (months) ‡	4 [1-5]	4 [2-6]	0.66
Other food allergy #	55.6	65.9	0.71
Associated asthma #	55.6	85.4	0.07
Tolerated dose during DBPCFC * (mg of milk proteins) ‡	-	34 [17-170]	-
Serum casein- specific IgE levels (kU/L) ‡	1 [0.5-2.7]	16.2 [5.5-60]	<0.01
Serum casein- specific IgG4 levels(mgA/L) ‡	0.3 [0.7-14]	2.9 [1.2-10.9]	0.4
Serum casein- specific IgE/IgG4 ratio (kU/mgA) ‡	1.1[0.2-3.3]	5 [2.6-10.5]	0.01
Serum α lactalbumin-specific IgE levels (kU/L) ‡	0[0-1.3]	3.8[1.4-10.7]	0.01
Serum β lactoglobulin- specific IgE levels (kU/L) ‡	0.7[0.2-1.1]	2.8[0.8-66.5]	0.06

* DBPCFC = double blind, placebo controlled food challenge

** NT group = natural-tolerant group. These children were not included because their threshold of tolerance during DBPCFC was above 2720 mg of milk protein

*** OIT = oral immunotherapy

‡ Median [interquartile range], for not normal continuous parameters

Normality was checked using Shapiro-Wilk test. Wilcoxon-Mann-Whitney test was used to compare continuous parameters between groups.

Results expressed in percentages. Comparison between groups was performed for categorical variables using Chi2-test if conditions met, or by Fisher-exact test otherwise.

¥ p-value was considered significant if <0.05.

Supplementary Table 4 – Comparison of safety outcomes at home according to the 2 arms of oral immunotherapy

	“High-risk” arm* n=18	“Low-risk” arm* n=23	p-value [¥]
Patients experiencing at least one AR** ‡	11, 61.1	12, 52.2	0.57
Patients experiencing multiple ARs ‡	4, 22.2	8, 34.8	0.38
Patients experiencing severe reactions***‡	4, 22.2	7, 31.8	0.73
Patients experiencing AR** with Astier score ≥ 3	4, 22.2	7, 31.8	0.73
Patients requiring epinephrine use‡	2, 11.8	3, 14.3	1

* “High-risk” arm is defined as the cluster schedule starting with raw milk, “low-risk” arm as the slow-progression schedule starting with baked-milk.

** AR= allergic reaction

*** Allergic reactions were considered severe if Astier score ≥ 3 and/or if an epinephrine injection was needed.

‡ Results expressed in absolute value and percentages (n,%). Comparison between groups was performed for categorical variables using Chi2-test if conditions met, or by Fisher-exact test otherwise.

¥p-value was considered significant if <0.05.

Supplementary material 1. Ancillary study on predictive factors of successful OIT

The aim of the ancillary analysis was to study the effects of various parameters as risk factors for epinephrine use at home, independently of time. A multivariate logistic regression was performed for this safety end-point, using a forward selection method¹. Clinically relevant variables with a $p < 0.15$ in the univariate analysis were tested in the multivariate logistic regression model^{2,3}. Results were expressed as odds ratio (ORs) and 95% confidence intervals (95% CIs).

Results

According to univariate analysis, children who required an epinephrine injection at home had a significantly higher casein-specific IgE/IgG4 ratio at inclusion ($p < 0.01$) and more frequently needed an epinephrine injection during the DBPCFC ($p = 0.04$) than children who did not (67% versus 8%). Multivariate logistic regression analysis confirmed baseline casein-specific IgE/IgG4 ratio (OR 1.11; IC95%: 1.01-1.22; $p = 0.03$) and epinephrine injection during the DBPCFC (OR 39.1; IC95%: 1.18-133; $p = 0.04$) as risk factors for subsequent epinephrine use at home. Asthma was not identified as a risk factor for epinephrine use at home.

Discussion

The potential success of an OIT protocol should be considered in terms of a risk/benefit ratio⁴. We have found three groups of patients, as previously described in other studies, and especially one group (accounting for approximately 1/3 of the OIT group) who did not respond to OIT⁵. While Martorell *et al* obtained better results than ours, they excluded children with a previous history of anaphylaxis shock due to CM⁶.

It is crucial to identify a priori a non-responder to avoid involving a patient in a long, unnecessary and potentially dangerous process. Another particular concern is that severe AR

could occur independently from the up-dosing phase, and is not always associated with cofactors⁷.

It would be expected that children with the lowest threshold of reactivity are more likely to benefit the most from OIT, protecting them from low-threshold anaphylactic reactions⁸.

However, only a few studies have focused on children with a low threshold of reactivity⁸.

Our findings regarding clinical predictive factors of severe AR during OIT are similar to those of Vazquez-Ortiz *et al*⁹ and Levy *et al*¹⁰. In these studies, initial severity of AR at inclusion was related to the risk of severe AR at home and increased the risk of unsuccessful OIT.

While the casein-specific IgE/IgG4 ratio has been described as a predictive factor of tolerated dose during an OIT protocol¹¹, it has not previously emerged as a risk factor of severe AR even though it is known that serum IgE and IgG4 levels are related to the threshold of reactivity¹².

Wood *et al* have also described risk factors which could identify high-risk subjects, such as a history of asthma and high baseline milk-specific IgE levels¹². Elizur *et al* have also shown that patients with asthma have a higher risk of more severe reactions and are less likely to reach full desensitization during OIT¹⁴. One explanation for the discrepancy observed with our results may be that our cohort corresponds to a highly specific group of patients with a high rate of associated asthma (85.4%). This high frequency of asthma in our cohort may make asthma a less discriminating criterion to differentiate the patients in terms of risk of AR. Overall, the rate of epinephrine use in our population was similar to that found in Elizur *et al*'s population of asthmatic patients.

Thus, there is still a need for reliable clinical factors and biomarkers that distinguish these subjects at high-risk of anaphylactic events from the others.

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