

ORIGINAL ARTICLE

Impact of dietary regimen on the duration of cow's milk allergy: a random allocation study

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Summary

Background The impact of diet on cow's milk allergy (CMA) duration and whether exposure to residual amounts of cow's milk protein influences the onset of tolerance are unknown.

Objective To prospectively assess the dietary factors influencing disease duration in a randomized cohort.

Methods We randomly switched the formula of symptomatic patients from the Milan Cow's Milk Allergy Cohort to one of three treatment groups according to the quarterly rotation of rice hydrolysate formula, extensively hydrolysed cow's milk formula and soy-based formula. In this intention-to-treat, randomized analysis, a hazard ratio (HR) estimation model was used to analyse dietary impact on disease duration.

Results Seventy-two children aged a mean of 14.1 ± 8.6 months at diagnosis were followed up for a median of 26 months. Fifty-one reached tolerance at a mean of 34.1 ± 15.2 months. The mean duration of disease was 40.2 ± 4.8 months with milk hydrolysate, 24.3 ± 3.6 months with rice and 24.3 ± 2.6 months with soy. Dietary choice independently predicted shorter duration of disease [adjusted HRs 3.09 ($P = 0.007$) for rice, 2.54 ($P = 0.02$) for soy, both against milk hydrolysate]. In 50 children not co-sensitized to soy, diet choice impacted the duration of disease more strongly [adjusted HRs 8.02 ($P = 0.006$) for rice, 6.53 ($P = 0.015$) for soy, both against milk hydrolysate].

Discussion Patients not exposed to cow's milk protein residue achieve cow's milk tolerance earlier than patients who follow an extensively hydrolysed cow's milk diet. This may be due to residual antigenicity in hydrolysed milks. As the effect of dietary intervention is stronger in patients not sensitized to soy, we infer that when atopic disease has progressed to multiple sensitizations, the elimination of allergenic exposure may not be sufficient to reduce the duration of CMA.

Keywords children, cow's milk allergy, cow's milk tolerance, dietary treatment, duration model

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Introduction

IgE-mediated hypersensitivity to cow's milk is predominantly a disease of early childhood, which is usually outgrown within 3–5 years [1]. Clinical and epidemiological predictors of the duration of cow's milk allergy (CMA) have been described [1–7] but whether dietary intervention affects the duration of disease remains unclear. The question has mostly been addressed retrospectively [8]. CMA is mostly treated by the avoidance of dairy products [9], but finding a nutritionally adequate, safe and palatable substitute for cow's milk to suit individual

patients can be difficult. In the absence of prospective, randomized intervention studies, the choice of formula for the management of CMA remains largely empirical within the framework of consensual treatment guidelines recommending the use of extensively hydrolysed cow's milk [10] or intact soy proteins (in children at least 6 months old) [11] as the first line of treatment. In Europe, two studies have proposed hydrolysed rice protein as a substitute [12,13].

The mainstay of clinical management remains the prescription of a hypoallergenic formula, but generalizing from different treatment outcomes from various

formulations can also be difficult, because co-sensitization to soy or residual antigenicity of even an extensively hydrolysed formula must also be considered. Secondary sensitization (to soy and cow's milk hydrolysate) has been investigated in the course of a large cohort study [14], but the role of continued exposure to subclinical amounts of allergenic proteins has not hitherto been evaluated with respect to the duration of CMA in infants and children. To address these issues, we prospectively evaluated the impact of dietary factors on the duration of CMA by following up the population of the earlier Milan Cow's Milk Allergy Cohort (MiCMAC) study [7].

Methods

From 1 January 2001 we prospectively carried out a concurrent investigation of the MiCMAC I population, a cohort of referred infants and children enrolled to determine prognostic predictors of tolerance. MiCMAC II was planned as a randomized, intention-to-treat analysis using the time to onset of tolerance among treatment groups to determine the influence of dietary regimen on the duration of CMA. The final date was 30 June 2007, when the sample size became sufficient for statistical power in the Cox regression model (82% power; 0.05 significance level; $N = 112$).

Study population and entry criteria

Three hundred and thirty-nine children referred to this institution had been evaluated by 31 December 2005. Independent predictors of duration of disease among the 112 participants were reported in the MiCMAC I study published in 2008 [7]. In the present study, MiCMAC II, we considered 72 patients receiving a formula that was different from the one prescribed by their paediatrician before referral for the evaluation of the continuing symptoms (entry criterion), having excluded 40 of the 112 patients because there was no clinical justification for altering their prescribed regimen, because 31 tolerated a soy-based formula (SF), six an extensively hydrolysed formula (eHF) and three a hydrolysed rice formula (HRF). Follow-up continued to 31 July 2007.

At their first referral visit to the clinic, all underwent a complete allergy work-up to confirm IgE-mediated CMA. Children positive in the skin prick test (SPT) (cut-off 3 mm weal diameter) and/or with a measurable cow's milk-specific IgE (cut-off 0.35 kUI/L; ImmunoCAP, Phadia, Uppsala, Sweden) were scheduled for a diagnostic double-blind, placebo-controlled food challenge (DBPCFC) for enrolment. Patients who were still symptomatic on their current regimen were switched to a different diet before undergoing the diagnostic DBPCFC (Fig. 1). A nutritional assessment was also performed.

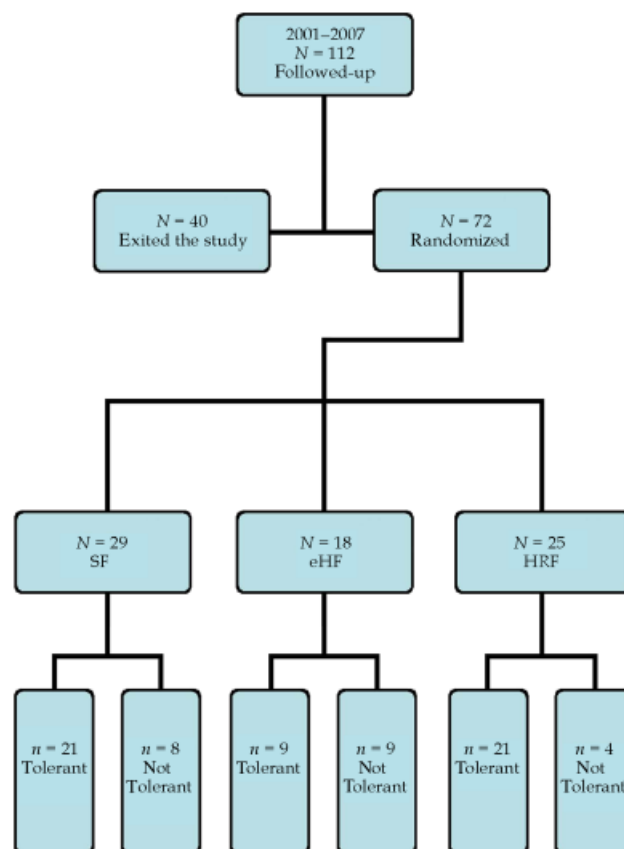


Fig. 1. The Milan Cow's Milk Allergy Cohort study patient flowchart.

Study regimens and random assignment

Children who were symptomatic at entry, including those partially breastfed [15,16], were allocated to one of three treatment groups. According to the current best practice, patients received one of the following:

- Children diagnosed in January–April received a soy formula (IsomilTM, Ross Laboratories, Columbus, OH, USA, or Humana SLTM, Humana Italia, Italy; SF).
- Children diagnosed in May–August received a cow's milk extensive hydrolysate (AlfareTM, Nestlé, Switzerland, or NutramigenTM, Mead Johnson Laboratories, Glenview, IL, USA; eHF).
- Children diagnosed in September–December received a rice hydrolysate (RisolocTM, Heinz, Italy; HRF).

The choice of these branded products and assignment to a particular regimen were made according to the date when the patient presented. In Italy, institutional statutes and fair trade rules require that competing brands of milk substitutes of equivalent nutritional and therapeutic requirements (according to the current Ministry of Health licences) must be prescribed on a periodic rotation basis unless there are individual clinical contraindications or a

documented history of reaction to the proposed alternative diet. Patients meeting the entry criteria were thus assigned to one of three study samples with equal probabilities of effect/failure of dietary influence and independent of subjective judgement. Thus, our study is quasi-randomized and falls within the descriptors of CONSORT recommendation procedures for the purpose of the analysis [17]. An elemental formula (Neocate[®], SHS, Liverpool, UK) was administered as rescue therapy if a patient clinically reacted to the prescribed regimen following confirmation of sensitization at SPT and/or specific IgE determination and allergy at a double-blinded challenge if the case warranted. We did not use an amino acid formula. Solid foods were introduced on the basis of assessment of individual food sensitivities. Breastfeeding was encouraged, and a paediatrician and a paediatric dietician designed a dietary schedule tailored to the mother and the child [15].

Oral food challenge method and follow-up

Participants underwent follow-up visits every 3 months (± 14 days) [7]. Six-monthly oral food challenges were repeated until 18 months after a diagnostic challenge and on a yearly basis thereafter. Accidental ingestion of milk without a clinical reaction prompted us to bring forward the scheduled oral food challenge. If accidental ingestion did produce clinical symptoms, the reported dose ingested was recorded. In this case, convincing symptoms were considered as substitutive of positive oral food challenges and the scheduled follow-up was delayed for 6–12 months.

Oral food challenge consisted of the administration of cow's milk in seven incremental doses of 0.1, 0.3, 1, 3, 10, 30 and 100 mL at 30-min intervals for 3.5 h under double-blinded conditions. Neocate[®] was used as a placebo and the *verum* was blinded following the guidance of EAACI [16]. Oral food challenge was discontinued at the first onset of symptoms. The method was targeted on immediate CMA and was thus limited to reactions occurring within 6 h.

Statistics

Kaplan–Meier curves after stratification for dietary option were computed. A Cox model was used for estimation of hazard ratio (HR) including patient risk factors identified in the previous study [7]. In the present statistical model, higher HRs correspond to a shorter disease duration. *P*-values below 0.05 were considered as statistically significant. All tests were two-sided, and were performed using SAS statistical package version 8.2 from SAS Institute (Cary, NC, USA).

Results

One hundred and twelve children referred to this institution had been evaluated in MiCMAC I by 31 December 2005. MiCMAC II is the follow-up of the 72 patients (24 girls, 48 boys) who were switched to a different dietary regimen (Fig. 1). The baseline characteristics of this sample are reported in Table 1. Allergic symptoms reported immediately after milk ingestion were anaphylactic shock ($n = 9$), asthma ($n = 14$), lip oedema ($n = 12$), generalized urticaria ($n = 45$), rhinitis ($n = 6$) and laryngeal stridor ($n = 5$). Fifteen patients presented with two, two with three symptoms.

The mean age at the first diagnostic challenge was 14.1 ± 8.6 SD (range 2–37) months. In this phase, the challenge was stopped after the immediate onset of asthma in 24 patients, lip oedema in 32, generalized urticaria in 34 and rhinitis in 12. Despite caution in stopping challenge at the first onset of symptoms, one patient experienced anaphylaxis and 29 were recorded with two symptoms. Although factors associated in MiCMAC I with a longer disease duration were consistently more frequent in the group assigned to eHF in MiCMAC II, no significant inter-group differences regarding prognostic factors for a longer duration of CMA were found upon multivariate analysis (data not shown).

Children were followed up for a mean period of 26.8 ± 17.2 and a median of 26 (range 6–65) months. Fifty-one patients achieved tolerance during the study. Among the 21 patients who were still symptomatic, the last diagnostic challenge determined asthma in four cases, lip oedema in eight, generalized urticaria in eight and rhinitis in one case.

In the whole sample, the median duration of disease estimated by Kaplan–Meier curve analysis was 28 (range 18–36) months and the mean estimated by Kaplan–Meier

Table 1. Baseline intergroup comparison in MiCMAC II (NS, non-significant)

Diet (no. of patients)	Formula		
	Soy (29)	eHF (18)	HRF (25)
Milk SPT mean diameter	7.34 $\pm$ 4.74	7.56 $\pm$ 4.16	5.6 $\pm$ 2.87
Soy co-sensitization: <i>N</i> (%)	11 (37.9)	7 (38.9)	4 (16)
Atopic dermatitis	22 (76%)	18 (100%)	20 (80%)
Asthma/rhinitis	11 (38%)	9 (50%)	8 (32%)
Patients reaching tolerance	21	9	21
Milk SPT mean diameter	6.62 $\pm$ 3.67	7.33 $\pm$ 3.74	5.24 $\pm$ 2.93
Soy co-sensitization	7 (33%)	5 (55%)	3 (14 %)
Patients not achieving tolerance	8	9	4
Milk SPT mean diameter	9.25 $\pm$ 6.76	7.78 $\pm$ 4.76	7.5 $\pm$ 1.73
Soy co-sensitization	4 (50%)	2 (22%)	1 (25%)
Intergroup comparison (<i>P</i>)	ns	ns	ns

MiCMAC, Milan Cow's Milk Allergy Cohort; eHF, extensively hydrolysed formula; HRF, hydrolysed rice formula; SPT, skin prick test.

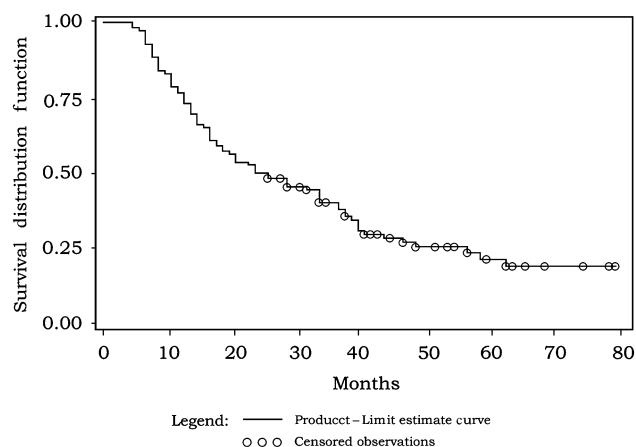


Fig. 2. Duration of disease in the Milan Cow's Milk Allergy Cohort study updated to 31 July 2007 (Kaplan-Meier survival analysis).

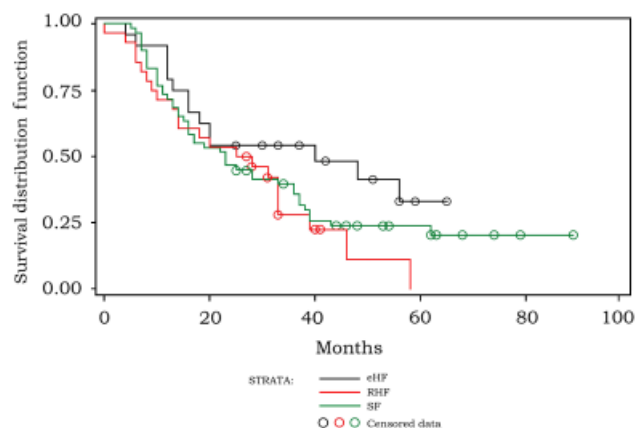


Fig. 3. Cow's milk substitutes and onset of tolerance in the Milan Cow's Milk Allergy Cohort study population ($N=72$) (Kaplan-Meier survival analysis). eHF, extensively hydrolysed formula; RHF, rice hydrolysed formula; SF, soy formula.

curve analysis was 30 ± 2.4 months ($\pm$ SE) (Fig. 2). Patients who reached tolerance did so on average at 34.1 ± 15.2 (10–78) months ($\pm$ SD). The mean age at the last positive challenge among the 19 children who failed to reach tolerance by the end of the study was 57.0 ± 14.6 SD (range 34–93) months. The data of the 40 patients who were not switched to a different regimen were examined for differences in demographic and clinical parameters with a possible bearing on the onset of tolerance but no significant patterns emerged (data not shown).

Type of formula and duration of cow's milk allergy

Figure 3 shows the Kaplan-Meier curves for duration of the disease stratified according to the type of prescribed formula. There was a significant difference between the three curves after 20 months ($P=0.018$; log-rank test). The estimated median disease duration, i.e. time before toler-

ance was achieved, was 56 months [95% CI not applicable] (mean: 40.2 ± 4.8 SE months) in the eHF group, 20 [95% CI 10–33] (mean: 24.3 ± 3.6 SE) months in the RHF group and 28 [95% CI 11–37] (mean: 24.3 ± 2.6 SE) months in the SF group. Table 2 shows the comparison of the effect of regimen on the duration of disease. HRF or SF, but not eHF, generated highly significant HR values predictive of a shorter duration of CMA. Comparison of HRF with SF did not yield significant HR. Both findings were confirmed after adjusting for other factors in multivariate analysis. Breastfeeding did not significantly alter inter-group differences in disease duration (data not shown).

The role of co-sensitization to soy

As soy sensitization emerged from the first MiCMAC data set [7] as an independent predictor of later achievement of tolerance to cow's milk, we stratified patients on the basis of soy co-sensitization to investigate the possible effect of this clinical feature on the duration of CMA. Twenty-two children had been sensitized to soy as indicated by SPT and/or ImmunoCAP. Kaplan-Meier analysis revealed the effect of diet in the 50 patients not sensitized to soy ($P=0.006$; log-rank test), as follows: in those receiving eHF, the estimated disease duration was 47.6 ± 6.4 SE months (central tendency and confidence limits were not calculated for this group because the intercept of the curve was not within our temporal observation), whereas children allocated to HRF presented with a median duration of CMA of 16 [95% CI 9–33] (mean 20.9 ± 3.2 SE) months and patients receiving SF were similar: median 16.5 [95% CI 8–39] (mean 21.3 ± 3.7 SE) months. Thus, for these 50 patients, HR values predictive of shorter disease (8.02 for

Table 2. Impact of dietary regimen on cow's milk allergy duration [higher hazard ratio (HR) predicts a shorter duration]

Variable	HR	95% CI		<i>P</i>
Whole population ($N=72$)				
HRF vs. Soy	1.32	0.72	2.42	0.37
HRF vs. eHF	3.01	1.35	6.71	0.007
Soy vs. eHF	2.29	1.03	5.08	0.04
HRF vs. Soy*	1.22	0.66	2.24	0.53
HRF vs. eHF*	3.09	1.37	6.97	0.007
Soy vs. eHF*	2.54	1.13	5.71	0.02
Not sensitized to soy ($N=50$)				
Soy vs. eHF	6.53	1.44	29.68	0.015
HRF vs. eHF	8.02	1.82	35.31	0.006
Soy-sensitized ($N=22$)†				
Soy vs. eHF	0.81	0.25	2.63	0.729
HRF vs. eHF	0.96	0.22	4.27	0.958

*Adjusted for factors identified as relevant for onset of tolerance in reference [7].

† χ^2 -test *P* for the interaction of soy sensitization with treatment = 0.095. eHF, extensively hydrolysed formula; HRF, hydrolysed rice formula.

HRF and 6.53 for SF) were obtained. This effect did not apply in the soy-sensitized group (Table 2). Thus, HRF and SF formulas promote tolerance only in children not sensitized to soy (Fig. 4).

Discussion

Our main finding is that infants and children with CMA who receive hydrolysed rice or a soy-based formula for the dietary management of their condition achieve tolerance significantly earlier than their peers on an extensively hydrolysed cow's milk formula. In this series, the median disease duration was almost three times longer for children in the group receiving eHF than in those receiving HRF (56 and 20 months, respectively), and twice as long (56 vs. 28 months) as in those receiving SF. In comparisons with eHF, the duration model applied to rice and soy formulas generated elevated HR values predicting faster tolerance. In MiCMAC I, the two factors influencing disease duration in the multivariate analysis were weal diameter at SPT with fresh cow's milk and soy co-sensitization as assessed by skin and blood tests [7]. Correction for these potential confounders in the present study confirmed the HR values found in the duration model analysis.

To the best of our knowledge, this study is the first to provide random-allocation data supporting the hypothesis that continued exposure to cow's milk protein residues could prolong the duration of CMA. Recently, an epidemiologic review of risk factors and theories regarding the development of food allergy explored the potential

prevention of allergy through early oral exposure [18]. In animal models, high doses of allergen orally administered early in life are tolerance inducing and prevent subsequent IgE-mediated responses. There are also epidemiological data indicating that high intakes of a specific food are associated with low incidence of allergy to the same food later in childhood. In an epidemiological study in Britain of the general paediatric population, children exposed to food allergens before their 16th week ran a significantly lower risk of sensitization and food allergy. However, almost all children who developed CMA had been exposed to cow's milk before their 12th week of life [19], suggesting that for a subpopulation of allergy-prone children, early exposure is not tolerogenic. Our data suggest that in the population of CMA referrals the continued exposure of CMA-allergic children to residual cows' milk protein delays the onset of tolerance.

We conclude that there are two subpopulations with different allergy phenotypes. In the general paediatric population, where an atopic predisposition is not expected to predominate, allergen exposure seems to have a tolerogenic effect [20], whereas in a specific population of sensitized and allergy-prone children, early exposure to the allergen induces sensitization.

Another conclusion is that multiple sensitization both predicts longer duration of CMA and nullifies the effect of dietary intervention. Co-sensitization to soy is frequent in CMA [21], but it had hitherto been assumed that it was not clinically relevant, as few co-sensitized patients actually express a true phenotypical reaction to soy [22,23]. However, we found that soy co-sensitization (which occurred in > 30% of the MiCMAC cohort) strongly influences the course of disease: indeed, the benefit of diet switching was completely abrogated by co-sensitization to soy (Fig. 4). Similarly, in the German MAS and the earlier Isle of Wight studies, a sub-population of patients expressed an early poly-sensitization pattern associated with earlier symptoms and more persistent and severe disease [24,25]. In our case load, a longer CMA duration mirrors the persistent wheezing found by these two studies. The data from MiCMAC I and II are thus both consistent with the emergence of a specific phenotype, characterized by enhanced indices of atopy associated with a longer duration of CMA and non-responsive to dietary intervention. As our study does suffer from the limitations of a small sample size, further epidemiological and especially gene expression studies are needed to determine whether a tolerance-prone (or persistence-skewed) phenotype can be distinguished in selected or unselected populations.

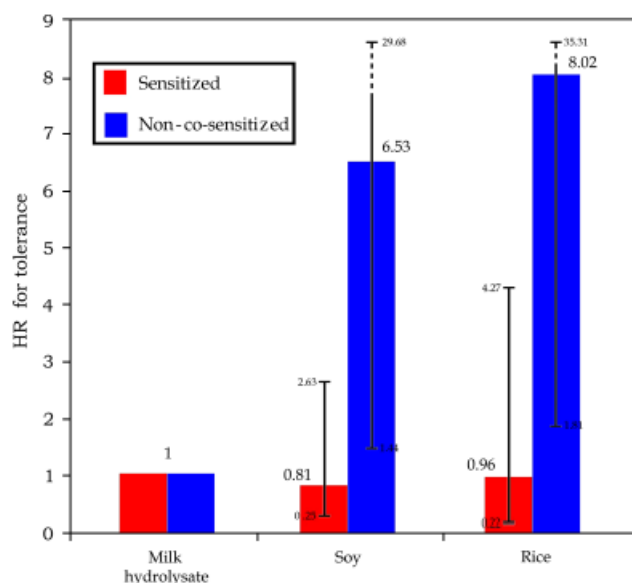


Fig. 4. Recapitulation of the duration model hazard ratios for cow's milk protein hydrolysate, soy formula and rice hydrolysate (Cox's regression analysis) showing the contrast between being (red column) and not being (blue column) co-sensitized to soy.

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