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RESEARCH ARTICLE

Lacticaseibacillus rhamnosus and breastmilk are associated with a decreased risk of atopic dermatitis in very low birth weight premature infants

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Abstract

In this study, we analyse the influence of nutrition during the early neonatal period on the development and prevention of atopic dermatitis (AD) in children with a history of very low birth weight (VLBW). A retrospective cohort study was performed of VLBW preterm infants to assess the risk of their developing AD during childhood, according to nutrition with breastmilk and/or probiotic supplementation during the neonatal period. The analysis focused on nutritional and early childhood follow-up data for 437 newborns, of whom 184 received probiotics up to 36 weeks postmenstrual age. AD was present in 23.5% of the study sample. Of the children who did not develop AD, 44.9% had received probiotics from birth to 36 weeks of gestational age. Therefore, the administration of probiotics to infants at less than 36 weeks postmenstrual age is associated with a protective effect against the development of AD (odds ratio (OR) 0.57; 95% confidence interval (CI) 0.34-0.93). Moreover, a protective interaction was observed between probiotic administration and breastmilk (OR 0.46; 95% CI 0.25-0.82). The adjusted data in the regression model allow us to observe a statistically significant association with the protective effect of *Lacticaseibacillus rhamnosus* with the development of AD at school age (OR 0.55; 95% CI 0.30-0.99). Probiotic supplementation in VLBW newborns is associated with a decreased risk of subsequent development of AD. Breastmilk strengthens the protective effect of probiotics against the development of AD.

Keywords

probiotics – human breastmilk – newborn – very low birth weight – atopic eczema

1 Introduction

Atopic dermatitis (AD) is one of the most common chronic childhood diseases, affecting approximately 10-20% of children in Europe (Nutten, 2015). AD is a chronic recurrent inflammation of the skin that leads to increased permeability and loss of the skin barrier, with an increased risk of allergic sensitisation (Fanfaret *et al.*, 2021). In recent years, there has been a significant increase in the prevalence of atopic diseases. The pathophysiology of these diseases is fundamentally affected by the metabolic activity resulting from alterations in the intestinal microbiota, as intestinal dysbiosis precedes the appearance of AD (Trikamjee *et al.*, 2020).

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The development of AD is also affected by environmental exposure and by genetic and epigenetic conditions that can influence the maturing immune system (Martinez and Guerra, 2018; Uberos, 2020). In this respect, a hygiene hypothesis has been proposed (Fuleihan, 2002), according to which contact with certain bacteria in the first years of life can reduce the subsequent risk of atopic disease (Pincus *et al.*, 2010). Other studies have considered the association between infectious factors and the development of atopic disease, but the results obtained, mainly related to the age of the child, are inconsistent (Rzehak *et al.*, 2013; Simpson *et al.*, 2020). In a longitudinal study of 47,015 children, Nagasaki *et al.* (2022) reported that the protective effect of early exposure to infectious agents, preventing the development of atopic disease, is maintained throughout the paediatric period.

Intestinal microbiota and their alterations (dysbiosis) in premature newborns are associated with diseases such as necrotising enterocolitis (NEC) and late-onset sepsis. The early administration of probiotics can help prevent their appearance (Iwatani *et al.*, 2014; Olsen *et al.*, 2016; Samuels *et al.*, 2016; Uberos *et al.*, 2022). In the long term, intestinal dysbiosis has been linked to inflammatory, metabolic, neurological and gastrointestinal diseases (Cuna *et al.*, 2021). During the neonatal period, the administration of breastmilk is recommended, among other reasons due to its microbiological and macromolecule content, such as human milk oligosaccharides (HMOs), which promote the growth of *Bifidobacterium* spp. (Damm *et al.*, 2017). However, in very low birth weight (VLBW) premature newborns, admitted to neonatal intensive care units (NICU), a significant proportion of breastmilk is provided by nasogastric tube as donated mother's milk (after being pasteurised) or as breastmilk previously frozen and administered after thawing (Altobelli *et al.*, 2020).

Breastmilk is indisputably preferable to formula feeding for VLBW newborns, as it both provides nutritional and immunological benefits and also strengthens the mother-infant bond. However, predictive studies seeking to detect an association between breastmilk and the (non-)development of AD have obtained inconsistent results. Moreover, very limited data are available in this respect on VLBW newborns.

The aim of the present study is, focusing on VLBW infants, to assess the associations between nutritional factors and AD during the early neonatal period, and their possible relationship with its prevention.

2 Material and methods

Study design

Retrospective cohort study composed of all newborns with a gestational age (GA) ≤32 weeks and/or birth weight ≤ 1500 g. born at our hospital between January 2009 and December 2021. The study goal was to evaluate the risk of their developing AD and the association between this risk and neonatal nutrition with breastmilk and/or probiotic supplementation.

A total of 520 VLBW infants were initially included in the study cohort. Of these, 65 died and a further 18 were excluded due to a lack of data (usually because the parents had changed their residence), incomplete health history records or the presence of severe congenital anomalies. In every case, the parents/guardians gave permission for their child to be included in the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Provincial Bioethics Committee of Granada (Spain), (Verification code: b1072175150294535cb06bd 44a5ca9ddfe619d24). Written informed consent was obtained from all subjects/patients.

Nutritional management

The nutritional strategy applied, including fluid intake, was in accordance with the standard protocol applied in the neonatal unit and with the recommendations of the Nutrition and Metabolism Group of the Spanish Neonatology Society (Narbona *et al.*, 2014; Uberos *et al.*, 2017). Because many of these infants were clinically unstable or had impaired enteral tolerance, much of the nutritional support during the first week of life was delivered parenterally. The normal procedure during the first days of life was to complement enteral with parenteral nutrition when complete enteral nutrition could not be established. The daily requirements of liquids, proteins and lipids were calculated daily. In all cases, the aim was that during the first week of life the minimum nutritional requirements to ensure growth should be met, in accordance with standard recommendations (Thureen, 2007). Enteral nutrition was administered via a nasogastric or orogastric tube. In accordance with our standard protocol, preference was given to feeding with fresh breastmilk. When this was not possible, frozen breastmilk or (if the mother authorised it) donated breastmilk was supplied. The mother's frozen breastmilk was warmed in a bain-marie and stirred before each feeding. The donated milk was pasteurised following the Holder method (62 °C for 30 min and cooling); a microbiolog-

ical control was then performed, after which the milk was labelled and stored for up to three months.

The fortification procedure was the same for both types of milk. Fortification was started at one week of chronological age and when enteral nutritional intake exceeded 80 ml/kg. Breastmilk was fortified using 4 g of PreNan Human Milk Fortifier (Nestlé, Zaragoza, Spain) per 100 ml of breastmilk. This product contains 3.6 g/100 Kcal of partially hydrolysed cow's milk whey proteins. Only when human milk was not available was preterm formula milk administered (Narbona *et al.*, 2014).

Protocol for the administration of probiotics

In our NICU, neonatologists have discretionally used probiotics since December 2013, in one of two formulations that showed good results in the prophylaxis of NEC in the preterm newborn (Uberos *et al.*, 2017a): *Lacticaseibacillus rhamnosus* ATCC 53103 (10^9 cfu/day) or *Lactobacillus acidophilus* ATCC 4356 (10^9 cfu/12 h) + *Bifidobacterium bifidum* ATCC 15696 (10^9 cfu/12 h) in accordance with the guidelines published by the Nutrition and Neonatal Metabolism Group of the Spanish Society of Neonatology (Narbona *et al.*, 2014). Until that date, probiotics were not used as NEC prophylaxis in our NICU. The probiotic supplementation, started at the first enteral feed and was continued until 35 weeks PMA or until the infant was discharged from the NICU. The prescription of one or other of these probiotics was determined according to the preferences of the relevant physician in the Neonatal Unit.

Atopic dermatitis

The diagnosis of AD is made clinically, based on historical features, the morphology and distribution of skin lesions, and associated clinical signs. These may include the presence of dry skin, red to brownish patches on the body, small raised bumps which may leak fluid when scratched, thickened cracked scaly skin, and raw, swollen skin from scratching. One of the earliest and most widely used sets of diagnostic criteria is the 1980 proposal by Hanifin and Rajka, which requires that three of four major criteria and three of twenty-three minor criteria be met (Rudzki *et al.*, 1999). In our study, the diagnosis was made by a dermatologist, it was reviewed by a project investigator that the Hanifin and Rajka criteria were met.

Statistical considerations

The descriptive data were summarised using medians and interquartile intervals for the continuous variables and distribution frequencies for the categorical variables. Quartiles of the Z-score of the difference between birth weight and weight at 36 weeks' GA were calculated. Univariate comparisons were made by the Mann-Whitney U test for the continuous variables and by the chi-square test for the categorical variables. The association of comorbidities in VLBW newborns and supplementation with one of the above-described probiotics was evaluated by multinomial regression analysis, adjusting for the variables differed according to the homogeneity analysis of the study groups, the adjustment was considered for those predictor variables that improved the regression model with narrower 95% confidence interval (CI) of the odds ratio (OR). A multivariate logistic regression analysis with forward stepwise selection (likelihood ratio) was performed. All statistical analyses were performed using IBM SPSS 20.0 for Windows software (IBM, Armonk, NY, USA).

3 Results

AD was observed in 23.5% of our sample of VLBW preterm infants. In this cohort, the mean ages of the children were 8.1 (4.8-10.5) years and and the mean age at diagnosis of AD was 5.7 (2.7-6.8) years. Prevalence data show a slight female preponderance. 36.8% of the children with a history of atopy in parents or siblings developed AD, compared to 22.1% among the whole sample who did not. In the study period considered (2009-2021), no adverse effects or complications derived from the administration of probiotics were observed, specifically no *L. rhamnosus* was isolated in blood culture, nor did NEC > stage II develop with identification of *L. rhamnosus* in peritoneal fluid.

Table 1 shows the weights and Z-scores obtained at birth, during the first week of life and at week 36 PMA. Fenton tables were used to calculate the Z-scores (Fenton and Kim, 2013). In our analysis, small-for-gestational age (SGA) is defined as birthweight below the 10th percentile for GA (Levine *et al.*, 2015) and extrauterine growth restriction is defined as poor weight gain at 36 weeks PMA, calculated as the difference between birth weight Z-score and Z-score for body weight at 36 weeks above the 75th percentile.

Of the 437 VLBW infants in the study sample 103 (23.5%) subsequently developed AD (see Figure 1). Among this cohort of 437 infants, 184 received probiotics until week 36 of postmenstrual age (PMA). Of these, 61 were given *L. acidophilus* + *B. bifidum* and 123 *L. rhamnosus* (Table 1). Table 1 also shows the characteris-

TABLE 1 Gestational and neonatal characteristics (<1,500 g)[2]

Characteristics	Without AD (n = 334)	With AD (n = 103)	*P*-value
Maternal	n (%)	n (%)	
PIH	38 (10.3)	10 (9.7)	0.63
Chorioamnionitis	38 (11.3)	12 (11.6)	0.75
Antibiotics	123 (36.8)	38 (36.8)	0.99
Glucocorticoids	293 (87.5)	90 (92.4)	0.92
PPROM	78 (25.6)	22 27.8)	0,74
Gestation (w)[1]	30 (28, 31)	30 (29, 32)	0.01
Gestation ≤27 w	76 (22.7)	9 (8.7)	0.002
Twin birth	136 (40.7)	45 (43.6)	0.60
Caesarean section	259 (77.5)	79 (76.6)	0.94
Family history of allergy/atopy	74 (22.1)	38 (36.8)	0.002
Neonatal			
Birth weight (g)[1]	1229 (992, 1444)	1297 (1077, 1481)	0.07
Birth weight (z-score)[1]	–0.44 (–1.06, 0.28)	–0.65 (–1.23, 0.01)	0.09
Weight 7 days (z-score)[1]	–1.15 (–1.58, –0.66)	–1.14 (–1.68, –0.67)	0.37
Weight PMA 36 w (g)[1]	2140 (1910, 2340)	2106 (1783, 2305)	0.61
Weight PMA 36 w (z-score)[1]	–1.42 (–2.00, –1.01)	–1.52 (–2.27, –1.12)	0.51
Height PMA 36 w (cm)[1]	46 (44.0-48.0)	45 (42.0-47.5)	0.11
Height PMA 36 w (z-score)[1]	–0.50 (–1.40, 0.40)	–1.00 (–2.10, 0.15)	0.12
CRIB index[1]	2 (1, 5)	2 (1, 3)	0.17
Male gender	176 (52.6)	46 44.6)	0.12
Apgar ≤5 (5 min)	35 (10.4)	8 (7.8)	0.46
SGA	63 (18.8)	21 (20.3)	0.67
Extrauterine growth restriction	136 (40.7)	48 (46.6)	0.90
Human breastmilk	212 (63.4)	65 (63.1)	0.85
Donor human milk (banking)	31 (9.2)	7 (6.7)	0.43
Premature milk formula	91 (27.2)	31 (30.1)	0.57
Breastmilk fortification	178 (53.3)	59 (57.3)	0.55
Central venous catheter (d)[1]	9 (2, 18)	7 (0, 16)	0.24
Age at full feeds (d)[1]	11 (8, 21)	10 (7, 21)	0.20
Early parenteral nutrition	161 (48.2)	42 (40.7)	0.18
Start enteral nutrition (d)	2 (2, 4)	2 (2, 4)	0.81
Parenteral nutrition (d)[1]	11 (7, 20)	10 (5, 19)	0.21
Probiotics	150 (44.9)	34 (33.9)	0.03
L. acidophilus + *B. bifidum*	49 (15.8)	12 (11.6)	0.40
L. rhamnosus	101 (29.6)	22 (22.3)	0.07
NEC			
Stage 1	38 (11.3)	11 (10.7)	0.86
Stage 2	22 (6.6)	9 (8.7)	0.49
Stage 3	8 (2.4)	1 (0.9)	0.38

1 Median (IQR). *P*-value X^2 for qualitative analysis, Mann-Whitney for quantitative analysis.

2 AD = atopic dermatitis; NEC = necrotising enterocholitis; PMA = postmenstrual age; PIH = pregnancy-induced hypertension; PPROM = preterm pre-labour rupture of membranes; SGA = small for gestational age.

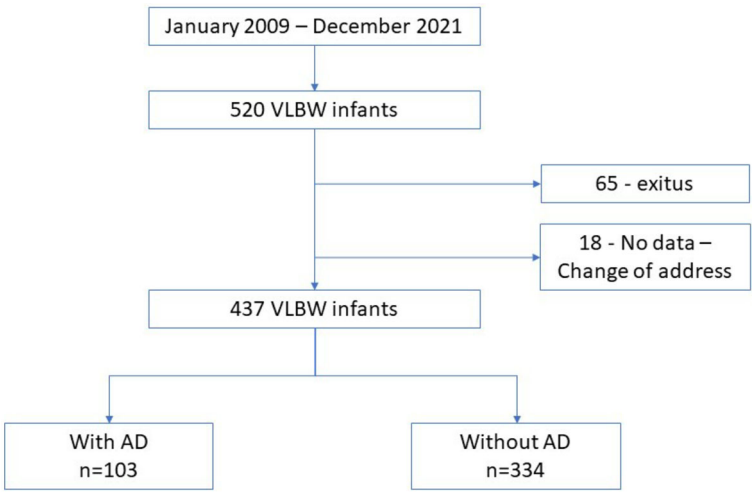

FIGURE 1 Flow diagram for the very low birth weight newborns included in the study. AD = atopic dermatitis.

tics of the maternal and neonatal variables in the above groups (i.e. with the presence or absence of AD). As it is expected that we observe more cases of family history of atopy/allergy in newborns with AD, this circumstance is considered in the adjustments of the regression models.

Among the cases with GA ≤27 weeks, significant differences between these groups were observed. Notably, AD was less prevalent in large preterm infants. 44.9% of the infants who did not develop AD had received probiotics from birth to 36 weeks of GA, compared to 33.9% of those presenting this condition. Table 2 shows the logistic regression analysis performed to determine the existence or otherwise of an association between the neonatal nutritional variables considered and the subsequent development of AD. In this respect, a family history of allergy/atopy and GA ≤27 weeks was taken as an adjustment variable and was found to be heterogeneously distributed between the two groups (Table 1). Extrauterine growth restriction was observed in 40.7% of the infants who did not develop AD and in 46.6% of those who did. Intrauterine growth restriction (i.e. SGA), on the other hand, did not differ significantly between the groups (18.8 vs 20.3%, respectively) (Table 1).

Table 2 shows the logistic regression models constructed, between the neonatal nutritional variables and the development of AD (dependent variable). Among other results, probiotics administered before 36 weeks PMA were significantly associated with a lower prevalence of AD (OR 0.57; 95% CI 0.34-0.93).

Table 3 shows the results of the analysis of logistic regression models for AD with interactions. Based on forward selection (likelihood ratio), all the variables listed in Table 2 and their interactions are included in the model. We observed a significant association between the appearance of AD and the interaction of probiotics with breastmilk (OR 0.46; 95% CI 0.25-0.82). Another significant association was observed with gestational age ≤ 27 weeks in the same regression model (OR 2.34; 95% CI 1.02-5.36). Table 4 shows the association analysis of the probiotic strains used with the appearance of AD, highlighting the statistical significance of the combination of *L. rhamnosus* (OR 0.55 (95% CI 0.30-0.99). However, there was no significant association with *L. acidophilus* + *B. bifidum* (OR 0.67; 95% CI 0.31-1.44).

Energy and protein intake during the early neonatal period did not differ between the groups with or without AD (Table 5). Nor were differences apparent regarding weight gain or growth at 36 weeks PMA (Table 1).

TABLE 2 Logistic regression of nutritional variables for atopic dermatitis

	OR (95% CI) Unadjusted	OR (95% CI) Adjusted[2]
Probiotics	0.61 (0.38-0.97)*	0.57 (0.34-0.93)*
Human breastmilk	0.95 (0.57-1.59)	0.81 (0.46-1.42)
Donor human milk (banking)	0.71 (0.30-1.67)	0.46 (0.19-1.09)
Age at full feeds	0.98 (0.96-1.00)	1.00 (0.97-1.02)
Parenteral nutrition	0.98 (0.96-1.00)	1.00 (0.97-1.03)
Start enteral nutrition	0.97 (0.90-1.05)	1.00 (0.92-1.08)

1 We observed a significant association of probiotics administered in the neonatal period on the development of atopic dermatitis at school age (protective effect).

2 OR = odds ratio; CI = confidence interval. Adjusted for gestation ≤27 weeks and family history of allergy/atopy. *= $P < 0.05$.

TABLE 3 Logistic regression models for atopic dermatitis with interactions[1,2]

	Wald	OR (95% CI)	*P*-value
Probiotics by human breastmilk	6.76	0.46 (0.25-0.82)	0.009
Gestation ≤27 w	4.09	2.34 (1.02-5.36)	0.04

1 Forward selection of stepwise regression (likelihood ratio). The adjusted regression model shows a significant interaction of probiotic intake up to week 36 post-menstrual age and breastfeeding with the development of atopic dermatitis at school age.

2 Variables not included in the forward stepwise regression model: Probiotics, Human breastmilk, Family history of allergy/atopy, Age at full feeds, Parenteral nutrition, Donor human milk (banking), Start enteral nutrition, Start enteral nutrition by probiotics; Family history of allergy/atopy by probiotics.

TABLE 4 Multinomial logistic regression for probiotic strains used in very low birthweight infants and atopic dermatitis[1]

	Wald	OR (95% CI)	*P*-value
Lactobacillus acidophilus + *Bifidobacterium bifidum*	1.03	0.67 (0.31-1.44)	0.30
Lacticaseibacillus rhamnosus	3.95	0.55 (0.30-0.99)	0.04

1 The adjusted data in the regression model allow us to observe a statistically significant association with the protective effect of *L. rhamnosus* with the development of atopic dermatitis at school age. Adjusted for gestation ≤27 weeks, Human breastmilk and family history of allergy/atopy.

TABLE 5 Median (p50) and interquartile range (IQR) of nutritional supports (enteral and parenteral) in the first week of life in children with and without atopic eczema[1]

Characteristics	Without AD (n = 334)	With AD (n = 103)	*P*-value
Energy (Kcal/kg/w)			
Enteral	50 (17-112)	56 (21-119)	0.40
Parenteral	381 (314-457)	387 (327-463)	0.89
Total	461 (401-516)	473 (403-518)	0.46
Carbohydrates (g/kg/w)			
Enteral	5.2 (1.8-11.8)	5.2 (1.9-12.9)	0.47
Parenteral	64.0 (54.4-71.2)	65.3 (57.3-74.2)	0.50
Total	70.3 (63.8-78.3)	73.5 (66.8-77.5)	0.07
Proteins (g/kg/w)			
Enteral	1.06 (0.3-2.6)	0.95 (0.3-2.4)	0.82
Parenteral	15.8 (10.1-19.3)	15.5 (10.6-18.0)	0.31
Total	17.3 (13.0-19.8)	16.3 (12.7-19.2)	0.40
Lipids (g/kg/w)			
Enteral	2.6 (0.80-5.83)	2.5 (1.07-6.05)	0.45
Parenteral	7.5 (4.5-10.5)	8.5 (3.5-10.5)	0.88
Total	12.5 (8.3-15.1)	12.1 (9.8-15.4)	0.27

1 AD = atopic dermatitis. We did not observe differences in preterm infants with and without AD and the macronutrient intakes administered to preterm infants during the first week of life.

Extrauterine growth restriction at 36 weeks PMA was not associated with the later development of AD (OR 1.26; 95% CI: 0.48-3.32).

4 Discussion

Our study shows that probiotics and in particular *L. rhamnosus* administered as a prophylaxis against NEC in VLBW newborns are associated with a decreased risk of subsequent AD. This effect is increased when the probiotics are combined with non-donor breastmilk nutrition.

The prevalence of AD in our cohort of VLBW premature infants was higher than that reported by Steffensen *et al.* (2000), who observed a prevalence of 3% among children weighing <2,500 g at birth. Olesen *et al.* (1997) found there was an increased risk of AD in post-term infants at 6.5 to 9.5 years after birth while David and Ewing suggested that preterm birth reduces the subsequent risk of AD (David and Ewing, 1988). Our results show that the prevalence of AD in VLBW preterm infants is lower than that reported elsewhere for a cohort of term infants (Kalliomaki *et al.*, 2001). Panduru *et al.* (2014) although they did not consider VLBW infants in their review, noted that low birth weight may be a protective factor against the development of AD.

The absorption of intact antigens in newborns with <33 weeks GA can be up to 100 times higher than in term newborns. For these periods, it is feasible that the absorption of large amounts of antigenic material could lead to the induction of tolerance rather than sensitisation (David and Ewing, 1988).

Several studies have shown that breastmilk helps protect the newborn against the development of AD, an association that is greater when there is a history of atopy in first-degree relatives and is less consistent in the absence of this family association (Gdalevich *et al.*, 2001). However, a meta-analysis conducted by Yang *et al.* (2009) found no strong evidence of any protective effect of breastmilk against the development of AD. In this respect, our own study differentiates between the administration of breastmilk and that of donated (pasteurised) human milk, in the understanding that digestive and neurological immaturity weakens the sucking and swallowing reflex, meaning that feeding by nasogastric tube becomes necessary. In addition, food intake must often be adjusted to small aliquots, which makes it necessary to freeze breastmilk until it is required. Our study shows that breastmilk interacts with probiotics administered up to week 36 PMA to achieve a protective effect on the development of AD at school age. HMOs patterns were found not to be affected by pasteurisation and freeze-drying of donor milks (Hahn *et al.*, 2019) which would explain the interaction effect detected in our study between consumption of human milk administered on most occasions after freezing and administration of probiotics.

In the VLBW newborn, the initial bacterial colonisation is essential for the maturation of the innate immune system. The predominance of lactobacilli (Zeng *et al.*, 2020) and bifidobacteria in the intestinal microbiota of the newborn is due, at least in part, to the existence of HMOs that favour their development (Sherman *et al.*, 2009). In the absence of these beneficial microbes, intestinal dysbiosis may occur after intestinal colonisation by proteobacteria, many of which are pathogenic (Campos-Martinez *et al.*, 2022).

Damm *et al.* (2017) examined whether the use of *L. rhamnosus* 1×10^9 and *Bifidobacterium animalis* subsp. *lactis* BB12 1×10^9 per day from birth to hospital discharge, in children with <30 weeks GA, reduced the prevalence of AD, and found no significant differences in this respect. Niele *et al.* (2013) analysed early enteral supplementation with prebiotics (oligosaccharides) in preterm infants with <32 weeks GA and/or <1,500 g weight at birth, and observed no increased risk of subsequent AD among these groups. Kalliomaki *et al.* (2001), in a double-blind randomised clinical trial of term newborns, evaluated the usefulness of *L. rhamnosus* in the prevention of AD, concluding that the administration of probiotics during pregnancy and up to six months after birth halves the risk of the infant developing early atopic disease.

In a meta-analysis, Voigt and Lele (2022) evaluated the effect of *L. rhamnosus* used solely or in conjunction with other probiotics applied perinatally for the prevention or treatment of atopic disease in children. These authors identified eleven randomised, double-blind, placebo-controlled trials undertaken to determine the incidence of AD following the perinatal (i.e. prenatal and postnatal) provision of *L. rhamnosus* to full-term newborns. In a recently published meta-analysis (Fijan *et al.*, 2023) showed that strains of *Limosilactobacillus fermentum* were significantly more effective than strains of *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei* or *L. rhamnosus*. One thing to keep in mind is that this meta-analysis does not include studies carried out in preterm infants. Other authors (Bjorkander *et al.*, 2020) have reported that the presence of certain lactobacilli species in the infant gut may influence allergy-related parameters in the peripheral immune system,

thereby contributing to allergy protection. According to our results, this influence extends to VLBW premature newborns. Thus, we observed a significant association between the use of *L. rhamnosus* in VLBW infants and a lower prevalence of AD. Other combinations of probiotics (*L. acidophilus* + *L. bifidum*) did not present a significant association with AD in these infants.

In a randomised controlled experimental study, Cukrowska *et al.* (2021) reported that different strains of *L. rhamnosus* in combination with *L. casei* facilitate the control of AD in 151 children aged under two years. However, these findings were contradicted by Plummer *et al.* (2020) who conducted a randomised clinical trial with the primary aim of evaluating the efficacy of a combination of probiotics on the incidence of late neonatal sepsis in preterm infants. Their secondary aim was to measure the efficacy of *Bifidobacterium longum* subsp. *infantis BB-02* + *Streptococcus thermophilus TH-4* + *Bifidobacterium animalis* subsp. *lactis BB-12*, in the prevention of AD. In this respect, the authors observed no significant effects. However, the effect of probiotics is strain-dependent and the latter authors did not include *L. rhamnosus* in their formulation.

In our cohort, the administration of breastmilk alone did not present a significant association with AD, although we did observe an interaction (potentiation) effect of breastmilk with probiotics, which was associated with a lower prevalence of AD. A similar interaction effect between supplemented probiotics and human milk has also been suggested by Nolan *et al.* (2020), who associated this with the content of HMOs, which would favour the growth of lactobacilli and bifidobacteria in the colon.

According to Zachariassen (2013), neither the fortification of breastmilk nor increased energy intake in preterm infants is associated with an increased risk of atopic disease. Corroborating this finding, we observed no significant association between energy or protein intake in the early neonatal period and the subsequent risk of AD. Neither was there any significant association with intrauterine or extrauterine growth restriction (the latter being frequent in VLBW newborns, especially those who are SGA) (Lima *et al.*, 2014).

Multiple mechanisms have been proposed through which probiotics decrease atopy, including shifting the balance between Th1/Th2 leukocytes toward Th1 through inhibition of Th2 cytokines or increasing the production of regulatory cytokines, such as interleukin-10, through of the maturation of dendritic cells or their receptors (Navarro-Lopez *et al.*, 2018).

The main limitation of our study, which is based on a retrospective observational design, is precisely the absence of randomisation, which increases the possibility of selection bias, we want to emphasise this fact. However, we believe it highly interesting to determine the repercussions of actions taken and treatments applied during the immediate postnatal period of VLBW premature newborns.

5 Conclusions

Probiotics are a promising resource in the prevention of allergic diseases, including AD. Supplementation with probiotics and, in particular, with *L. rhamnosus* in VLBW infants is associated with a decreased risk of AD. Breastmilk reinforces the protective effect of probiotics against the development of AD later in life.

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Authors' contribution

JU designed the analysis and interpretation of data, wrote the article and critically reviewed it for important intellectual content. He approves the version to be published and agrees to be responsible for all aspects of the work and to ensure that questions related to the accuracy or completeness of any part of the work are properly investigated and resolved. A. C-M, N. T-C, R. S-N, E. F-M and A. G-C made a substantial contribution to the conception, design and writing of the article and critically reviewed it for important intellectual content. They both approve this version to be published and agree to be responsible for all aspects of the work to ensure that questions related to the accuracy or completeness of any part of the work are properly investigated and resolved. A. R-L and A. G-C made a substantial contribution to the acquisition of data and to writing the article and critically reviewed it for important intellectual content. They approve the version to be published and agree to be responsible for all aspects of the work to ensure that questions related to the accuracy or

completeness of any part of the work are properly investigated and resolved.

Conflict of interest

The authors have no financial relationships relevant to this article to disclose. The authors have no relevant conflicts of interest to declare.

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Statement of ethics

The authors affirm that the work is original and is not being evaluated in any other journal.

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