ORIGINAL ARTICLE

Arquivos Brasileiros de Oftalmologia

Association between human breast milk and retinopathy of prematurity

Associação entre ingesta de leite materno e desenvolvimento de retinopatia da prematuridade

Luciana Teixeira Fonseca[1,2], Denise C. Senna[1], Gabriela Unchalo Eckert[3], Rita de Cássia Silveira[2,3], Renato Soibelmann Procianoy[2,3]

1. Hospital da Criança Conceição, Porto Alegre, RS, Brazil.
2. Department of Pediatrics, Neonatology Section, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
3. Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.

ABSTRACT | Purpose: To evaluate the possible protective effect of breast milk against retinopathy of prematurity by comparing the amount of breast milk received by patients who developed retinopathy of prematurity and those who did not and to determine both the required minimum amount of breast milk and the time of life during which neonates need to receive breast milk for this effect to be significant. **Methods:** Cohort study of newborns with a birth weight of <1500 g or gestational age of <32 weeks, or both, born between January 2011 and October 2014 and hospitalized within the first 24 h of life in the Hospital Criança Conceição Neonatal Intensive Care Unit in Porto Alegre, RS, Brazil. **Results:** The prevalence of retinopathy of prematurity of any degree was 31% (100 of 323 patients) and that of severe retinopathy of prematurity was of 9% (29 of 323 patients). The median amounts of breast milk received daily by patients with and without retinopathy of prematurity were 4.9 mL/kg (interquartile range, 0.3-15.4) and 10.2 mL/kg (1.5-25.5), respectively. The amount of breast milk received in the first 6 weeks of life was inversely associated with the incidence of both retinopathy of prematurity of any degree and severe retinopathy of prematurity in the univariate analyses. However, the statistical significance was maintained only during the sixth week of life in a per-period multivariate analysis controlling for confounding factors. **Conclusions:** Small amounts of breast milk are inadequate to prevent retinopathy of prematurity in premature newborns at risk for the disease.

Keywords: Retinopathy of prematurity; Milk, human; Breast feeding; Infant, very low birth weight; Insulin-like growth factor I; Infant, premature

RESUMO | Objetivos: Avaliar o possível efeito protetor do leite materno contra a retinopatia da prematuridade, através da comparação da quantidade de leite materno recebida entre os pacientes que desenvolveram retinopatia da prematuridade e aqueles livres da doença. Tentar determinar a quantidade mínima necessária e o momento em que o recém-nascido precisa receber o leite materno para que esse efeito seja significativo. **Métodos:** Estudo de coorte observacional incluindo recém-nascidos com peso de nascimento inferior a 1500 gramas e/ou com idade gestacional inferior a 32 semanas, nascidos no período de janeiro de 2011 a outubro de 2014 e internados nas primeiras 24 horas de vida na UTI Neonatal do Hospital da Criança Conceição em Porto Alegre. **Resultados:** A prevalência da retinopatia da prematuridade em qualquer grau foi de 31% (100 casos em 323 pacientes) e a de retinopatia da prematuridade grave foi de 9% (29 casos em 323 pacientes). A mediana da quantidade de leite materno recebida pelos pacientes foi de 10,2 mL/kg/dia entre os pacientes sem retinopatia da prematuridade (amplitude interquartil 1,5-25,5) e de 4,9 mL/kg/dia entre os pacientes com retinopatia da prematuridade (0,3-15,4). A quantidade de leite materno recebida nas primeiras seis semanas de vida foi inversamente associada à incidência de retinopatia da prematuridade em qualquer grau e de retinopatia da prematuridade grave nas análises univariadas, mas a significância estatística não se manteve após análise multivariada para controle de fatores confundidores na maioria dos períodos avaliados, exceto na sexta semana de vida. **Conclusão:** Pequenas quantidades de leite materno não são suficientes para prevenção de retinopatia da prematuridade em recém-nascidos com de risco para a doença.

Descritores: Retinopatia da prematuridade; Leite humano; Aleitamento materno; Recém-nascido de muito baixo peso; Fator de crescimento insulin-like I; Recém-nascido prematuro

Submitted for publication: June 20, 2017
Accepted for publication: November 15, 2017

Funding: No specific financial support was available for this study.

Disclosure of potential conflicts of interest: None of the authors have any potential conflict of interest to disclose.

Corresponding author: Luciana Teixeira Fonseca.

Av. Ferdinand Kisslinger, 80/1001B. Porto Alegre, RS - 91360-054 - Brasil
E-mail: lumteixeira@gmail.com

Approved by the following research ethics committee: Grupo Hospitalar Conceição (#13-185).

INTRODUCTION

Retinopathy of prematurity (ROP) is a vasoproliferative eye disease secondary to the incomplete vascularization of the retina in premature infants[1]. The prevalence of ROP has increased along with improvements in neonatal services in many countries. Because ROP can cause blindness[2], it results in social and financial burdens to society. Furthermore, irreversibly impaired vision also prevents suitable cognitive and psychomotor development in affected infants[3].

The etiology of ROP is multifactorial and remains to be fully elucidated. Factors involved in its pathogenesis include exposure of the developing retina to abnormal oxygen levels[4-6] and deficiencies in insulin-like growth fator-1 (IGF-1)[7]. Human breast milk (BM) contains IGF-1[8] and therefore may have a protective effect against the development of ROP.

The association between BM intake and ROP has been studied with conflicting results. Heller et al.[9] found no evidence that BM intake reduces the risk of severe ROP (surgically treated) in extremely low-birth-weight (ELBW) infants (BW <1000 grams). Hylander et al.[10] found that compared with very low-birth-weight (VLBW) infants (BW <1500 g) who consumed exclusively infant formula, those who consumed BM had a lower incidence of ROP. Kao et al.[11] compared the incidence of ROP in newborns who received any amount of BM (from their mothers or from a milk bank) and those who received only infant formula and found no significant association between the consumption of BM and ROP risk. Manzoni et al.[12] compared 314 newborns fed exclusively BM with 184 newborns who received only premature infant formula. The incidence of ROP at any stage was significantly lower in the newborns who consumed BM.

This variation in study results may be related to the amount of BM consumed by premature infants. The protective effect of BM may manifest only at higher ingestion levels or when BM is consumed at specific times, perhaps during the first weeks of life, which is when IGF-1 deficiency is involved in the development of ROP[7].

The purpose of this study was to assess the protective effect of BM against ROP by comparing the amount of BM ingested by infants who developed ROP and those who did not. The study also aimed to determine the minimum amount of BM required and the timing with which a newborn needs to receive BM for the effect to be significant.

METHODS

Population and logistics

The present study was an observational cohort study using retrospective and prospective data. The study population comprised newborns with a BW of <1500 g or a gestational age of <32 weeks, or both, who were hospitalized in the Hospital Criança Conceição (HCC) Neonatal Intensive Care Unit (NICU) between January 2011 and October 2014. Patients were excluded if they had major congenital malformations, died before completing the ophthalmic assessment, or arrived at the HCC from other hospitals after 24 h of life.

The retrospective data were collected using patient records from the HCC Medical Archive and Statistics Service. The prospective portion of the research was conducted by monitoring patients during their stays in the NICU and Intermediate Care Unit and, after discharge, during follow-up visits to the Ophthalmology Outpatient Clinic.

The following variables were evaluated: (1) delivery data including prenatal care, use of antenatal corticosteroids, presence of ovular infection, presence of preeclampsia, and mode of delivery; (2) newborn data, including sex, BW, gestational age (established through echography when obstetric ultrasound was performed up to 12 weeks of gestational age or with a Ballard score in the absence of early ultrasound), weight classification for gestational age (small, appropriate, or large), fifth-minute Apgar score, score for neonatal acute physiology, perinatal extension II), transfer from another hospital, and twinning; (3) newborn NICU evolution data, including the use of dopamine, duration of mechanical ventilation, duration of nasal continuous positive airway pressure use, total duration of oxygen use, number of transfusions of red cell concentrates during NICU hospitalization, duration of use of parenteral nutrition, age at which enteral feeding started, age at which the consumption of a full enteral diet was achieved, length of hospital stay, and the presence of respiratory distress syndrome, early- or late-onset sepsis, fungal sepsis (all sepsis diagnoses were confirmed with blood culture), hyperglycemia, necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), intraventricular hemorrhage grade III or IV, and persistent arterial duct; (4) feeding data including the amount of BM received as measured daily in milliliters per kilogram until the newborns completed 6 weeks of life or were discharged; (5) and ophthalmic assessment data including the development and severity of ROP and need for surgical treatment

using laser photocoagulation. Disease staging was performed according to the International Classification of ROP (International Committee for the Classification of ROP, 2005). Retina mapping tests are included in the routine care of the HCC Neonatology Service and were performed by a single trained ophthalmologist. Ophthalmic follow-up was initiated during hospitalization and maintained on an outpatient basis after discharge. The follow-up was considered complete when patients were monitored for up to 40 weeks of corrected gestational age or had fully vascularized retinas or a minimal avascular area.

This study was approved by the Research Ethics Committees at Grupo Hospitalar Conceição and Federal University of Rio Grande do Sul. The parents of the patients in the prospective arm of the study gave informed written consent for participation.

Statistical analysis

The calculated sample size was 292 patients based on an ROP incidence of 28% (local data), the aim to detect an ROP reduction of 50% with 80% power, and a level of statistical significance of 0.05. These statistical criteria were adopted from a study by Hylander et al.[10], demonstrating that VLBW newborns fed BM had an incidence of ROP lower than that of VLBW newborns fed exclusively infant formula. Initially, the patients were divided into two groups: those with ROP (of any degree) and those without ROP.

The quantitative variables were presented as means and standard deviation (symmetrical distribution) or medians and interquartile range (asymmetric distribution). The qualitative variables were described as absolute and relative frequencies.

Student's *t*-test (symmetrical distribution) or Mann-Whitney test (asymmetric distribution) was applied for the comparison of quantitative variables. For the evaluation of the association between qualitative variables, the chi-square and Fisher exact tests were used.

The analyses were performed by determining the amount of BM intake throughout the study period (first 42 days of life) and weekly (weeks 1-6). These data were used to evaluate the timing for BM intake that was effective for preventing ROP. A multivariate analysis was conducted using the Poisson regression with adjustment for robust variances to determine the prevalence ratios adjusted for the key factors involved. Some of the factors identified as significant in the univariate analysis were excluded from the multivariate analysis owing to multicollinearity.

Subsequently, the patients were divided into new groups: patients with severe ROP (grade ≥3) and patients without ROP or with mild ROP (grade ≤2). All of the analyses were repeated in these new groups. A p value of <0.05 was considered statistically significant, and the analyses were performed using SPSS version 18.0.

RESULTS

During the study period, 466 newborns with a BW of <1500 g or gestational age of <32 weeks, or both, were admitted to the HCC NICU within their first 24 h of life. Of these patients, 12 were excluded because of major congenital malformations and 112 died before completing the ophthalmic evaluation. A total of 342 patients were initially enrolled in the study. Nineteen of these patients were excluded because they did not complete the ophthalmic evaluation (missed outpatient follow-up appointments). The final analysis included 323 patients.

The prevalence of ROP of any degree was 31% (100 cases) and that of severe ROP was 9% (29 cases). During the first 42 days of life, the average daily BM intake was 13.7 mL/kg (19.1% of the total milk intake). The average daily intake of infant formula during this period was 57.9 mL/kg (80.9% of the total amount of enteral nutrition consumed).

In the initial analysis comparing patients with ROP of any degree and patients without ROP, the median daily BM intakes were 4.9 mL/kg (interquartile range, 0.3-15.4) and 10.2 mL/kg (1.5-25.5), respectively. In the univariate analysis, the presence of ROP had a statistically significant association with several of the variables studied, including BM intake during the first 42 days of life and, separately, during all 6 weeks evaluated (Table 1). In the multivariate analysis, only fungal sepsis, persistent arterial duct, gestational age, and BM intake at week 6 remained significantly associated with the development of ROP (Table 2).

In the second analysis comparing patients with severe ROP and patients without ROP or with mild ROP, the median daily BM intakes were 8.4 mL/kg (1.3-23.1) and 2.4 mL/kg (0.12-12.6), respectively. In the univariate analysis, the presence of severe ROP had a statistically significant association with several variables studied, including BM intake during the first 42 days of life and separately in weeks 1, 4, 5, and 6 (Table 3). In the multivariate analysis, only fungal sepsis, BPD, and mechanical ventilation time remained significantly associated with the development of severe ROP (Table 4).

Table 1. Comparison between patients included in the study and those who died before completing the ophthalmic evaluation

	Patients included 323 patients	Deaths 102 patients	P value
Prenatal care	295 (91.3%)	90 (88.5%)	0.496
Antenatal steroids	216 (66.9%)	43 (42.3%)	<0.001
Ovular infection	32 (10.0%)	23 (22.9%)	0.023
ROM >18 h	66 (20.4%)	16 (16.2%)	0.416
Preeclampsia	114 (35.3%)	25 (24.8%)	0.060
Male	162 (50.2%)	53 (52.4%)	0.776
Fifth-minute Apgar score <7	53 (16.4%)	57 (56.3%)	<0.001
Birth weight	1200.8 ± 264.3	785.1 ± 206.0	<0.001
Gestational age	30.1 ± 2.3	26.1 ± 2.8	<0.001
SNAPPE II	15 (8-29)	53 (40-70.5)	<0.001

Data expressed as means ± standard deviation, median and interquartile range, or in proportion (%) when applicable.
ROM= rupture of membranes; SNAPPE II= score for neonatal acute physiology, perinatal extension II.

Table 2. Association between the variables studied and ROP in the univariate analysis

	Newborns without ROP 223 patients (69%)	Newborns with ROP 100 patients (31%)	P value
Prenatal	206 (92.4%)	89 (89%)	0.425
Antenatal corticosteroids	152 (68.2%)	64 (64%)	0.544
Ovular infection	25 (11.2%)	17 (17%)	0.253
Preeclampsia	84 (37.7%)	30 (30%)	0.262
Vaginal delivery	74 (33.2%)	37 (37%)	0.581
Male gender	107 (48.0%)	55 (55%)	0.332
Small for gestational age	103 (46.2%)	34 (34%)	0.085
Transfer	17 (7.6%)	8 (8%)	0.923
Twinning	48 (21.5%)	15 (15%)	0.240
Fifth-minute Apgar score <7	23 (10.5%)	29 (29%)	<0.001
RDS	99 (44.4%)	86 (86%)	<0.001
Early onset sepsis	4 (1.8%)	1 (1%)	0.635
Late-onset sepsis	57 (25.6%)	57 (57%)	<0.001
Fungal sepsis	6 (2.7%)	12 (12%)	0.002
Use of dopamine	49 (22.0%)	44 (44%)	<0.001
Hyperglycemia	19 (8.5%)	17 (17%)	0.034
NEC	19 (8.5%)	19 (19%)	0.009
BPD	37 (16.6%)	57 (57%)	<0.001
IVH III/IV	5 (2.2%)	17 (17%)	<0.001
PDA	31 (13.9%)	47 (47%)	<0.001
Birth weight	1272 ± 237	1041 ± 253	<0.001
Gestational age	30.8 ± 2	28.6 ± 2.2	<0.001
SNAPPE II	13 (5-23)	25 (13-47.5)	<0.001
No. of RCC transfusions	1 (0-2)	5 (2-9)	<0.001
MV duration	0 (0-3)	9.5 (1-38.5)	<0.001
Nasal CPAP duration	3 (1-8)	8.5 (3-15)	<0.001
Supplemental O_2 days	5 (1-16)	35 (11-65.5)	<0.001
Parenteral nutrition time	3 (2-6)	6 (3.5-10)	<0.001
Enteral feeding start	4.7 (2-6)	6 (3.5-10)	<0.001
Full enteral feeding	16 (2-24)	28.5 (17-40)	<0.001
Length of hospital stay	48 ± 20	80 ± 39	<0.001
BM 42 days	10.2 (1.5-25.5)	4.9 (0.3-15.4)	0.005
BM week 1	0.43 (0-4.3)	0 (0-1.1)	0.003
BM week 2	7 (0-22.6)	1.4 (0-10.5)	0.003
BM week 3	5.9 (0-23)	2.2 (0-16.7)	0.034
BM week 4	7.9 (0-27.6)	1.4 (0-14.5)	0.015
BM week 5	6.9 (0-34.1)	1.3 (0-14.3)	0.003
BM week 6	4 (0-37)	0 (0-15)	0.016

Data expressed as means ± standard deviation, median and interquartile range, or as a rate (%) when applicable.
BM= breast milk; BPD= bronchopulmonary dysplasia; CPAP= continuous positive airway pressure; IVH III/IV= grade III or IV intraventricular hemorrhage; MV= mechanical ventilation; NEC= necrotizing enterocolitis; O_2= oxygen; PDA= patent ductus arteriosus; RCC= red cell concentrates; RDS= respiratory distress syndrome; ROP= retinopathy of prematurity; SNAPPE II= score for neonatal acute physiology, perinatal extension II.

Table 3. Variables significantly associated with ROP in the multivariate analysis

	Relative risk	95% CI	P value
Fungal sepsis	1.47	1.06-2.03	0.021
PDA	1.58	1.18-2.12	0.002
Gestational age	0.87	0.80-0.95	0.002
BM week 6	0.99	0.98-1.00	0.041

The following variables were also used in the multivariate analysis but did not maintain statistical significance: late-onset sepsis; necrotizing enterocolitis; bronchopulmonary dysplasia; grade III or IV intraventricular hemorrhage; score for neonatal acute physiology, perinatal extension II; and mechanical ventilation time.
BM= breast milk; CI= confidence interval; PDA= patent ductus arteriosus; ROP= retinopathy of prematurity.

DISCUSSION

In the present study, BM showed a protective effect against ROP in the univariate analysis during all periods evaluated; however, only BM intake during the sixth week of life maintained statistical significance after adjusting for confounding variables. The vast majority of premature infants at risk for ROP in our study population received a mixed diet that contained predominantly of infant formula and a very low mean amount of BM: only 13.7 mL/kg per day during the first 42 days of life

Table 4. Association between the variables studied and severe ROP in the univariate analysis

	Newborns without severe ROP 294 patients (91%)	Newborns with severe ROP 29 patients (9%)	P value
Prenatal	288 (91.8%)	25 (86.2%)	0.298
Ovular infection	37 (12.6%)	5 (17.2%)	0.560
Antenatal corticosteroids	202 (68.7%)	14 (48.2%)	0.029
Preeclampsia	109 (37.0%)	5 (17.2%)	0.044
Vaginal delivery	97 (33.0%)	14 (48.2%)	0.105
Male gender	146 (49.7%)	16 (55.2%)	0.571
Small for gestational age	127 (43.2%)	10 (34.5%)	0.433
Transfer	22 (7.5%)	3 (10.3%)	0.480
Twinning	59 (20.1%)	4 (13.8%)	0.623
5th-minute Apgar <7	40 (13.8%)	12 (42.9%)	<0.001
RDS	157 (53.4%)	28 (96.6%)	0.003
Early-onset sepsis	5 (1.7%)	0 (0%)	1.000
Late-onset sepsis	97 (33.0%)	17 (58.6%)	0.008
Fungal sepsis	11 (3.7%)	7 (24.1%)	<0.001
Use of dopamine	76 (25.9%)	17 (58.6%)	<0.001
Hyperglycemia	29 (9.9%)	7 (24.1%)	0.019
NEC	28 (9.5%)	10 (34.5%)	<0.001
BPD	69 (23.5%)	25 (86.2%)	<0.001
IVH III/IV	14 (4.8%)	8 (27.6%)	<0.001
PDA	62 (21.1%)	16 (55.2%)	<0.001
Birth weight	1230 ± 250	901 ± 211	<0.001
Gestational age	30.4 ± 2.2	27.5.6 ± 2.1	<0.001
SNAPPE II	15 (8-15)	39 (16.5-52.5)	<0.001
No. of RCC transfusions	1 (0-3.3)	10 (5.5-16.5)	<0.001
MV duration	0 (0-7)	49 (15.5-62.5)	<0.001
Nasal CPAP duration	3 (1-10)	9 (5-15)	0.028
Supplemental O_2 days	7 (2-26.3)	68 (44-91)	<0.001
Parenteral nutrition time	16 (10.8-23.6)	35 (20-60)	<0.001
Enteral feeding start	4 (2-7)	6 (4-12)	0.006
Full enteral feeding	17 (13-28)	34 (22-53.5)	0.020
Length of hospital stay	53 ± 23.6	106.8 ± 53.2	<0.001
BM 42 days	8.4 (1.3-23.1)	2.4 (0.12-12.6)	0.034
BM week 1	0 (0-3.6)	0 (0-1.3)	0.044
BM week 2	5.6 (0-21.3)	0 (0-7.1)	0.087
BM week 3	4.8 (0-21.2)	1.1 (0-15.7)	0.085
BM week 4	6.6 (0-25)	0 (0-6.6)	0.048
BM week 5	6 (0-28)	0 (0-10.9)	0.030
BM week 6	3.6 (0-30)	0 (0-4.2)	0.042

Data expressed as means ± standard deviation, median and interquartile range, or as a rate (%) when applicable.
BM= breast milk; BPD= bronchopulmonary dysplasia; CPAP= continuous positive airway pressure; IVH III/IV= grade III or IV intraventricular hemorrhage; MV= mechanical ventilation; NEC= necrotizing enterocolitis; O_2= oxygen; PDA= patent ductus arteriosus; RCC= red cell concentrates; RDS= respiratory distress syndrome; ROP= retinopathy of prematurity; SNAPPE II, score for neonatal acute physiology, perinatal extension II.

Table 5. Risk factors significantly associated with severe ROP in the multivariate analysis

	Relative risk	95% CI	P value
Fungal sepsis	3.64	1.57-8.41	0.003
BPD	4.20	1.28-13.82	0.018
MV duration	1.01	1.01- 1.02	<0.001

The following variables were also used in the multivariate analysis but did not maintain statistical significance: late-onset sepsis, gestational age and amount of BM received in the first 42 days of life. Nnecrotizing enterocolitis; grade III or IV intraventricular hemorrhage; score for neonatal acute physiology, perinatal extension II.
BM= brest milk; BPD= bronchopulmonary dysplasia; CI= confidence interval; MV= mechanical ventilation; ROP= retinopathy of prematurity.

(19.1% of the total milk intake during the period). This low BM intake is likely inadequate for the prevention of ROP.

The inadequate protective effect of BM against ROP observed in our study may also have been the result of BM processing. Most of the BM obtained at HCC is pasteurized. Only BM obtained at the bedside at the time of feeding is offered fresh (a practice that remains infrequent in our unit). All frozen BM used during the study period was both thawed and pasteurized, and the pasteurization process reduces the amounts of IGF-1 in BM. A study by Goelz et al.[13] showed that after 30 min of heating, the amount of IGF-1 in BM is reduced by 39.4%.

Furthermore, BM may be more effective in the prevention of ROP when intake occurs at a different time, such as after the sixth week of life. BM intake during the sixth week of life in our study had a protective effect against ROP despite the small amounts of BM consumption by the newborns in the cohort.

A 2013 study by Manzoni et al.[12] firmly established the protective effect of the exclusive feeding of BM (fresh or frozen and thawed) compared with the exclusive feeding of infant formula. A newly published study by Spiegler et al.[14] also compared VLBW newborns who received exclusively BM and those receiving exclusively infant formula and showed a greater risk for the development of BPD, NEC, and ROP in infants fed formula alone.

The conflicting results of previous studies may be due to differences in the amount of BM consumed by the newborns in the study populations. For example, Heller et al.[9] found no association between BM intake and reduced risk of ROP in ELBW infants who underwent surgery, but the BM intake in their study population was low, accounting for only approximately 15% of the total feeding volume during hospitalization. Hylander et al.[10] reported that compared with VLBW infants fed exclusively formula, those who consumed BM had a lower incidence of ROP. Notably, the breastfeeding rate among the mothers of these infants was 57%, which is quite high compared with the rate of 36% reported nationally in the United States.

A recently published meta-analysis by Zhou et al.[15] supported the hypothesis that BM consumption reduces the risk of ROP. The authors selected longitudinal studies comparing the incidence of ROP in newborns fed BM and those fed infant formula. For ROP at any stage, the odds ratios with 95% confidence interval were as follows: BM alone versus infant formula in any amount: 0.29 (0.12-0.72); predominantly BM versus predominantly infant formula: 0.51 (0.26-1.03); BM in any amount versus infant formula alone: 0.54 (0.15-1.96); and BM alone versus infant formula alone: 0.25 (0.13-0.49). For severe ROP, the results were 0.11 (0.04-0.30), 0.16 (0.06-0.43), 0.42 (0.08-2.18), and 0.10 (0.04-0.29), respectively. The authors concluded that the limited current evidence available suggests that BM intake plays a protective role in the prevention of both ROP at any stage and severe ROP in very premature newborns. The results of this meta-analysis clearly demonstrate the association between the amount of BM consumed and the level of protection against ROP. However, although this association is undeniable when BM feeding is exclusive, it is difficult to demonstrate when infants are fed a mixture of BM and formula.

Similar conclusions have been drawn regarding the importance of BM intake during the later perinatal period. Okamoto et al.[16] undertook a study of possible risk factors for the progression of ROP to retinal detachment in ELBW infants. Their results showed a substantial difference in the daily intake of BM in infants with and without retinal detachment when evaluated with respect to gestational age during the period of 5-7 weeks' postnatal age. Although IGF-1 deficiency during the first weeks of life is known to be related to ROP[7], other components in BM may also influence the development of ROP and contribute to a protective effect during later perinatal periods. Human milk contains long-chain polyunsaturated fatty acids and antioxidant enzymes, including inositol[17,18]. The poor antioxidant defenses of premature infants and their reduced ability to synthesize long-chain polyunsaturated fatty acids have been implicated in the pathogenesis of ROP[19, 20]. Moreover, inositol is an essential nutrient for the growth and survival of human cells, and its administration to preterm

infants results in a lower incidence of ROP[21]. The risk of developing severe ROP is higher in children with low serum inositol concentrations[22].

A positive aspect of our study was our attempt to overcome the methodological problems of previous studies by using an experimental design specifically aimed at identifying the amount of BM needed to prevent ROP in newborns at risk for the disease. Also notable is the successful follow-up of our cohort, in which only 19 of 342 patients (5.5%) were lost owing to a failure to complete the ophthalmic assessment at the outpatient clinic.

Our study is limited by the low BM intake of newborns at risk for ROP in our population. This limitation highlights the need to strengthen incentives for milking by mothers of preterm infants. Regardless of the amount of protection BM intake may offer against ROP, the feeding of BM in premature infants is known to reduce the risk of various negative outcomes of prematurity, including late-onset sepsis and NEC. BM intake also reduces the length of NICU hospitalization[23].

Although a randomized clinical trial would be the ideal experimental design for investigating the association between BM and the development of ROP, it would be unethical to give BM to one study group and not to another. Therefore, prospective cohort studies are the most appropriate option. Cohort studies analyze associations of exposure and effect by comparing the occurrence of diseases between patients exposed and not exposed to a risk factor or, as in the present study, a protective factor. This type of study may present confounding biases, which arise because of differences inherent in the probabilities of falling ill among exposed and unexposed populations. Because ROP has a multifactorial etiology that remains to be fully elucidated, its risk and protective factors are even more difficult to define. Compared with newborns who are smaller and more immature, newborns with higher BWs and greater gestational age have a lower risk of developing ROP yet tend to achieve a fuller enteral diet more quickly and, consequently, a higher amount of BM. In an attempt to minimize confounding factors, we performed a multivariate analysis in which the predictor variables were analyzed simultaneously and the effect of each variable was adjusted to that of the others.

The results of our study allow us to conclude that small amounts of BM intake are inadequate to prevent ROP. Future studies that investigate a possible cut-off point-that is, a minimum amount of BM intake that confers a protective effect against ROP-must include newborns who consume larger amounts of BM. Our study, despite its limitations, demonstrated a significant protective effect of BM against ROP in an analysis of BM intake during the sixth week of life. Therefore, per-period analyses that evaluate BM intake and ROP risk, particularly during later perinatal periods such as the fifth to eighth weeks of life, would be of special interest

REFERENCES

1. Fielder AR, Reynolds JD. Retinopathy of prematurity: clinical aspects. Semin Neonatol. 2001;6(6):461-75.
2. Quinn GE, Gilbert C, Darlow BA, Zin A. Retinopathy of prematurity: an epidemic in the making. Chin Med J (Engl). 2010;123(20): 2929-37.
3. Wheatley CM, Dickinson JL, Mackey DA, Craig JE, Sale MM. Retinopathy of prematurity: recent advances in our understanding. Br J Ophthalmol. 2002;86(6):696-700.
4. Campbell K. Intensive oxygen therapy as a possible cause of retrolental fibroplasia: a clinical approach. Med J Australia. 1951;2: 48-50.
5. Patz A, Hoeck LE, De La Cruz. Studies on the effect of high oxygen administration in retrolental fibroplasia: I. Nursery observations. Am J Ophthalmol. 1952;35:1248-53.
6. Patz A. The role of oxygen in retrolental fibroplasia. Albrecht Von Graefe's Arch Clin Exp Ophthalmol. 1975;195:77-85.
7. Hellstrom A, Perruzzi C, Ju M, Engstrom E, Hard AL, Liu JL, et al. Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. Proc Natl Acad Sci USA. 2001;98:5804-8.
8. Ozgurtas T, Aydin I, Turan O, Koc E, Hirfanoglu IM, Acikel CH, Akyol M, Erbil MK. Vascular endothelial growth factor, basic fibroblast growth factor, insulin-like growth factor-I and platelet-derived growth factor levels in human milk of mothers with term and preterm neonates. Cytokine. 2010;50(2):192-4.
9. Heller CD, O'Shea M, Yao Q, Langer J, Ehrenkranz RA, Phelps DL, Poole WK, Stoll B, Duara S, Oh W, Lemons J, Poindexter B. Human milk intake and retinopathy of prematurity in extremely low birth weight infants. Pediatrics. 2007;120(1):1-9.
10. Hylander MA, Strobino DM, Pezzullo JC, Dhanireddy R. Association of human milk feedings with a reduction in retinopathy of prematurity among very low birth weight infants. J Perinatol. 2001; 21(6):356-62.
11. Kao JS, Dawson JD, Murray JC, Dagle JM, Berends SK, Gillen SB, Bell EF. Possible roles of bilirubin and breast milk in protection against retinopathy of prematurity. Acta Paediatr. 2011;100(3):347-51.
12. Manzoni P, Stolfi I, Pedicino R, Vagnarelli F, Mosca F, Pugni L, Bollani L, Pozzi M, Gomez K, Tzialla C, Borghesi A, Decembrino L, Mostert M, Latino MA, Priolo C, Galletto P, Gallo E, Rizzollo S, Tavella E, Luparia M, Corona G, Barberi I, Tridapalli E, Faldella G, Vetrano G, Memo L, Saia OS, Bordignon L, Messner H, Cattani S, Della Casa E, Laforgia N, Quercia M, Romeo M, Betta PM, Rinaldi M, Magaldi R, Maule M, Stronati M, Farina D; Italian Task Force for the Study and Prevention of Neonatal Fungal Infections, Italian Society of Neonatology. Human milk feeding prevents retinopathy of prematurity (ROP) in preterm VLBW neonates. Early Hum Dev. 2013;89(1):64-8.
13. Goelz R, Hihn E, Hamprecht K, Dietz K, Jahn G, Poets C, Elmlinger M. Effects of different CMV-heat-inactivation-methods on growth factors in human breast milk. Pediatr Res. 2009;65(4):458-61.

14. Spiegler J, Preuß M, Gebauer C, Bendiks M, Herting E, Göpel W; German Neonatal Network GNN. Does Breastmilk Influence the Development of Bronchopulmonary Dysplasia? J Pediatr. 2015 Nov 25. [Epub ahead of print].
15. Zhou J, Shukla VV, John D, Chen C. Human Milk Feeding as a Protective Factor for Retinopathy of Prematurity: A Meta-analysis. Pediatrics. 2015;136(6):1576-86.
16. Okamoto T, Shirai M, Kokubo M, Takahashi S, Kajino M, Takase M, Sakata H, Oki J. Human milk reduces the risk of retinal detachment in extremely low-birth weight infants. Pediatr Int. 2007;49(6):894-7.
17. Jensen RG. Lipids in human milk. Lipids. 1999;34:1243-71.
18. Friel JK, Martin SM, Langdon M, Herzberg GR, Buettner GR. Milk from mothers of both premature and full-term infants provides better antioxidant protection than does infant formula. Pediatr Res. 2002;51:612-8.
19. Asikainen TM, White CW. Pulmonary antioxidant defenses in the preterm newborn with respiratory distress and bronchopulmonary dysplasia in evolution: implications for antioxidant therapy. Antioxid Redox Signal. 2004;6:155-67.
20. Connor WE, Neuringer M, Reisbick S. Essential fatty acids: the importance of n-3 fatty acids in the retina and brain. Nutr Rev. 1992; 50:21-9.
21. Hallman M, Bry K, Hoppu K, et al. Inositol supplementation in premature infants with respiratory distress syndrome. N Engl J Med. 1992;326:1233-9.
22. Friedman CA, McVey J, Borne MJ, James M, May WL, Temple DM, Robbins KK, Miller CJ, Rawson JE. Relationship between serum inositol concentration and development of retinopathy of prematurity: a prospective study. J Pediatr Ophthalmol Strabismus. 2000;37(2):79-86.
23. Schanler RJ1, Lau C, Hurst NM, Smith EO. Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. Pediatrics. 2005;116(2):400-6.