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Differential Gender Response to Respiratory Infections and to the Protective Effect of Breast Milk in Preterm Infants

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Abstract

OBJECTIVE—The protective role of breastfeeding against severe acute lung disease in infants is well established, but its mechanism is unclear. Most hypotheses assume that breastfeeding confers similar passive protection to every infant; however, a few observations have suggested that the benefits of breast milk against severe lung disease may differ according to gender. The objective of this study was to determine whether the effect of breastfeeding on susceptibility to severe acute lung disease among infants at high risk is different for girls and boys.

METHODS—A cohort was analyzed prospectively by use of 2 different strategies: (1) predictors of first episode of rehospitalization by univariate and multivariate analyses using robust Poisson regression and (2) mean number of rehospitalizations between groups using multiple regression negative binomial models.

RESULTS—A total of 119 high-risk, very low birth weight infants were enrolled. Breast milk protected girls but not boys against severe acute lung disease. The interaction between breastfeeding and gender was clinically and statistically significant, even after adjustment for variables that can

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What's Known on This Subject

The protective role of breastfeeding against severe acute lung disease in infants is well established, but its mechanism is unclear. Most hypotheses assume that breastfeeding confers similar passive protection to every infant.

What This Study Adds

This study reveals an unexpected gender-related difference in the protective effects of breast milk; suggests that severity of respiratory diseases in infancy may be amenable to modulation by a nonspecific mechanism; challenges the established dogma that the protective effect of breast milk is exerted by passive transfer of IgA; and contributes to redefine the populations of premature infants at highest risk for severe lung disease, in this case nonbreastfeeding girls.

affect severity of acute lung disease. Disease was most severe in formula-fed girls (versus formula-fed boys).

CONCLUSIONS—Breastfeeding decreased the risk for severe acute lung disease in girls but not in boys. These findings suggest that breast milk protection is not universally conferred by passive transfer of humoral immunity (which should be gender indifferent), show that respiratory symptoms may be amenable to nonspecific modulation, and identify nonbreastfed preterm infant girls as an at-risk group for severe acute lung disease.

Keywords

lower respiratory infection; breast milk; respiratory syncytial virus; prematurity; gender

Viral respiratory infections are the main pediatric cause of hospitalization in infants and young children during the winter worldwide.¹ In particular, very low birth weight (VLBW) infants are at high risk for severe viral lung disease.^{2–4} Although respiratory infections are a serious threat to VLBW infants everywhere, the rate of severe presentations is higher in infants who live in developing countries.^{4,5} Unlike in industrialized nations,^{2–4} preterm infants in developing countries often lack access to prophylaxis against respiratory syncytial virus (RSV)⁵ and have low immunization rates against influenza virus.⁶ Virus-specific preventive interventions against other agents are not available. Hospitalization rates for VLBW infants in developing countries can exceed 25% during the first year of life.^{5,7}

The protective role of breastfeeding against severe respiratory infections in healthy term infants is well established.^{8,9} In VLBW infants, a similar beneficial effect has been described.¹⁰ For all of these populations, the mechanism of breast milk–mediated protection is unclear. A widely accepted hypothesis attributes the effect of breast milk to neutralization of infectious agents by passively transferred secretory immunoglobulin A in the respiratory tract of exposed infants.^{9,11–13} Alternative theories attribute protection to the passive transfer of other molecules with anti-infective activity.^{14–16} All of these hypotheses assume that breastfeeding confers similar passive protection to each and every infant; however, a few observations in recent years suggested that the benefits of breast milk against acute lung disease may differ according to gender.^{17,18} If confirmed, then these gender differences could challenge the current theories that postulate a passive mechanism of breast milk–mediated protection and identify certain subgroups of infants who are at higher risk for severe disease (ie, those not protected by breast milk). We present the results of a prospective cohort study designed to determine the role of gender and breastfeeding on susceptibility to severe acute lung disease among intensively monitored infants at high risk.

METHODS

Patients

This prospective cohort study of infants and young children at high risk for pulmonary disease was conducted between June 1, 2003, and May 31, 2005, at the Garrahan Children's Hospital and the Maternidad Sarda High Risk Clinics in Buenos Aires, Argentina.⁵ Families of infants who were leaving the NICUs were invited to participate during their first visit to the clinics between June 1, 2003, and November 30, 2004. Written, witnessed informed consent was obtained from all parents or guardians. Families received oral and written instructions for recognizing respiratory signs and symptoms in special workshops at the time of infant discharge from the NICU or study enrollment. Participating parents or guardians were asked to visit the clinics every time their child developed changes in baseline respiratory status. Travel expenses to the clinic and a small meal allowance for accompanying siblings were provided for every visit, because the clinics monitor a population of low socioeconomic status (33% of

families below the poverty line). Participating children were examined in the clinics monthly, and families were contacted by telephone every 2 weeks by a pediatrician (Dr Klein) with a standard questionnaire to inquire about changes in respiratory status. The High Risk Clinics provide highly specialized care for the children that is not widely available in other public institutions in Buenos Aires, and infants were assigned a primary care provider on enrollment; therefore, patients were unlikely to receive additional care in other centers. When a participating child was admitted to a nonparticipating institution, however, a pediatrician (Dr Klein) visited him or her and the family during that hospitalization. Children who attended the participating High Risk Clinics did not receive a humanized monoclonal antibody against RSV (palivizumab) during the respiratory viral season, because of cost constraints.

Participating children had to be <6 months of corrected gestational age, have a birth weight of <1500 g, and reach the corrected gestational age of 1 year before May 31, 2005, to enroll in the study. Families had to live <70 km (~44 miles) away from the clinics. Children with <6 months of life expectancy, known bleeding disorders, immune deficiencies, or orofacial malformations were excluded from participation. Families who did not have a home telephone number were routinely contacted through scheduled telephone appointments at their neighbor's or a relative's home. The study was approved by the institutional review boards of all participating institutions.

Clinical Information

Breastfeeding was categorized as exclusive and nonexclusive. Exclusive breastfeeding was infrequent in our population (4 of 119), because most VLBW infants require supplemental nutrition. Duration of breastfeeding was examined monthly at every visit. Because World Health Organization recommendations emphasize the importance of unrestricted breastfeeding whenever the infant shows signs of hunger during the day or night¹⁹ (and, in some cases, these episodes are brief and intermittent), establishing the number of daily episodes of lactation was not possible.

Acute respiratory infection was defined as the sudden onset of ≥ 1 of the following signs or symptoms: rhinorrhea, pharyngitis, cough, retractions, wheezing, or crackles with or without fever. Severe acute lung disease was defined as need for rehospitalization (determined on the basis of changes in baseline oxygen requirement and the development of respiratory distress) and used as the main outcome variable for the study. An additional outcome variable for severity scored changes in pulmonary status during every visit (Table 1).²⁰ The evaluations were conducted by 1 of 3 pediatricians trained in the study protocol by using a modification of the validated score designed to detect changes in oxygen saturation, respiratory rate, and pulmonary signs in children at high risk.²⁰ This score discriminated upper from lower respiratory infections (Table 1). Infants were followed clinically as inpatients or outpatients until resolution of symptoms. Nasal secretions were assayed for RSV; human metapneumovirus (hMPV); human parainfluenza virus (hPIV) 1, 2, and 3; and influenza virus by reverse transcriptase–polymerase chain reaction by use of the Hexaplex Plus assay (Prodesse, Waukesha, WI.), as described previously.^{5,21}

Birth weight, gestational age, length of NICU stay, length of ventilatory support, mother's age, ≥ 1 family member smoking in the home, diagnosis of asthma in 1 or both parents, detection of viral RNA from nasal secretions, presence of siblings <10 years of age in the household, maternal education, household income, and presence and severity of bronchopulmonary dysplasia (BPD; oxygen supplementation for ≥ 28 days after birth²²) were considered as potential confounders and included in the multivariate analyses. Baseline severity for BPD was established at 36 weeks of gestational age.²²

Statistical Analysis

The main outcome used as an indicator of severe disease was rehospitalization. The main research question addressed in this article is whether the effect of breastfeeding on severe acute lung disease is different in girls and boys; therefore, we focused on assessing the interaction between breastfeeding and gender, adjusting for potential confounders. Because an infant can have >1 episode of hospitalization and these episodes are not independent for a given infant, 2 different analytical strategies were used. In the first strategy, we studied the first episode of acute respiratory disease (subsequent episodes were ignored in the analysis). The role of epidemiologic and clinical variables as independent predictors of severe disease was examined by univariate and multivariate analyses using robust Poisson regression to compute adjusted risk ratios.²⁴ The Kaplan-Meier method was used to compare time to first episode of rehospitalization between groups (ie, male and female), and differences were assessed by the log rank test. A Cox proportional hazard model was constructed to estimate the impact of risk factors and to control for confounders.²⁵

In the second analytical strategy, all episodes of respiratory infection and hospitalization were included in the analyses, and the mean number of events between groups were compared using multiple regression negative binomial models. The negative binomial distribution can accommodate the different propensities to severe disease across members of the population.²⁶

Frequencies of epidemiologic characteristics in children and hospital course of preterm infants divided according to gender were compared by χ^2 and Student's *t* test when appropriate. Statistical analyses were performed by using the Stata package for IBM-PC (Stata Corp, College Station, TX).

RESULTS

Study Population

Since June 2003, 208 VLBW infants attended the High Risk Clinics for the first time. Among these, 67 did not meet inclusion criteria or had exclusion criteria (61 did not reach the corrected gestational age of 1 year before completion of the study, 2 had HIV infection, and 4 resided >70 km away from the clinics). Of the 141 patients who met enrollment criteria, 9 parents did not consent to their infant's participation in the study, and 13 children were lost to follow-up; therefore, 119 infants at high risk participated in the cohort. Epidemiologic characteristics of participants and excluded infants were similar (data not shown).

Among the 119 participating infants, 40 (34%) were younger than 1 month of corrected age at enrollment, 92 (77%) were younger than 3 months, and 110 (92%) were younger than 5 months. Among infants with BPD, 11 (23%) had mild, 14 (30%) moderate, and 22 (47%) severe disease.

Clinical Characteristics

The characteristics of the study population of 119 pre-term infants are described in Table 2. Eighty-eight infants had a lower respiratory infection (LRI), with 46 infants experiencing moderate to severe symptoms and 33 requiring rehospitalization. Mean age at the time of the first respiratory infection was 3.1 ± 2.7 months. Sixty-seven percent of the episodes occurred during the respiratory viral season (May 1 through August 31),²¹ when RSV represented 48% of detections. During the study, RSV elicited 24 respiratory infections, hPIV3 was responsible for 15, hMPV was associated with 8, and influenza virus caused 6 infectious episodes. hPIV1 and hPIV2 were responsible for 1 infection each.

Gender and the Effect of Breast Milk on Respiratory Disease

Because an infant can have >1 episode of hospitalization and these episodes are not independent for a given infant, 2 different analytical strategies were used to determine the role of gender and breastfeeding on respiratory disease. Analysis focused on the first acute episode of respiratory infection after discharge from the NICU. There, the analysis showed that breast milk protected girls but not boys against rehospitalization (Table 3). The interaction between breastfeeding and gender was clinically and statistically significant. Adjusting the observed effect for epidemiologic and clinical variables that can affect susceptibility to respiratory infections did not alter the results (Table 3). To rule out any possibility of gender bias in the decisions to rehospitalize children, we also analyzed these effects using a previously validated clinical severity score (Table 1). The same protective effect of breast milk in girls but not in boys was observed by using the score, which had a strong correlation with rehospitalizations ($P < .0001$). The protective effect of breastfeeding for girls was conferred both by breastfeeding on enrollment (Table 3) and by breastfeeding at the time of the acute infectious episode (data not shown).

Figure 1 shows the risk for rehospitalization by gender and breastfeeding status. It is interesting that in addition to the gender-specific protective effect of breast milk, rehospitalizations were significantly more frequent among nonbreastfeeding girls than among nonbreastfeeding boys ($P = .01$; Table 3, Fig 1).

In the second analytical strategy, which used all episodes of respiratory infection and hospitalization, a similar pattern was observed (Table 4). Breastfeeding was strongly protective against rehospitalization for girls but not for boys.

Finally, because the frequency of social exposure can affect infection rates and consequently the rate of severe disease, we examined whether boys and girls were differentially infected by respiratory viruses. No differences in the frequency of respiratory infections were observed (Table 4).

DISCUSSION

In this prospective cohort of VLBW infants, breastfeeding decreased the risk for severe acute lung disease in girls but not in boys. Breastfeeding had a strong protective effect against severe disease in infant girls who experienced their first symptomatic respiratory infection, and this beneficial effect persisted throughout all episodes of respiratory infection during the first year of life.

These findings have important implications for pediatric research. First, they strongly suggest that breast milk protection against acute respiratory infections is not universally conferred by passive transfer of humoral immunity^{9,11–13} or a soluble molecule with anti-infective properties,^{14–16} which should be gender indifferent. In fact, breast milk–mediated maturation or activation of a critical protective pathway in the immune system or respiratory tract of girls seems likely. A better understanding of the protective components in breast milk is important for strategically improving maternal nutrition and/or supplementing alternative sources of nutrition for infants who receive mixed feedings or no breast milk worldwide.

Second, our findings show that symptoms elicited by a variety of respiratory viruses may be amenable to nonspecific modulation, resulting in a significant decrease in disease severity. Most of the current research efforts to prevent severe acute lung disease are focused on the development of vaccines or therapies against individual viruses.^{27–30} The potential impact of a nonspecific modulatory molecule that can improve clinical symptoms against many different agents cannot be overstated. In fact, because differences were detected in episode severity but

not in the frequency of infections, these observations suggest that on similar infections, the immune response and/or respiratory tract in children plays a critical role in disease symptoms.

Third, our study describes an increased susceptibility to severe acute lung disease in nonbreastfed infant girls compared with all other groups. In fact, all but 1 nonbreastfed girl had an LRI. Furthermore, despite representing only 17% of all infants in the study, nonbreastfed girls experienced 48% of all hospitalizations during the first episode of acute respiratory disease. These findings challenge the well-established paradigm that infant boys have worse acute lung disease than infant girls^{1–3} and suggest that differences in disease symptoms are determined by mediators other than just baseline lung function, which is comparatively decreased in VLBW boys.³¹ To date, no gender differences had been identified that could contribute to target better expensive interventions, such as palivizumab, in countries with limited resources. Redefinition of the relative risk for severe disease according to breastfeeding and gender may be an important tool for resource allocation in the future.

The mechanism of this different susceptibility to disease is unclear. Female-specific susceptibilities during infancy have been reported for other diseases. Increased mortality after immunization with a high titer measles vaccine was described for girls from countries of low socioeconomic status,^{32,33} and more severe disease was observed in girls compared with boys who had *Bordetella pertussis* infection³⁴. Both diseases, measles and pertussis, have been associated with a Th2 polarization of the immune response.^{35,36} It is interesting that enhanced severity of respiratory infections in infants has also been associated with a strong Th2 bias,^{37,38} which is common during the early months of life³⁹; therefore, gender-specific differences in Th bias may affect susceptibility to respiratory infections. Whether breastfeeding can also affect Th polarization should be investigated.⁴⁰ Alternatively, the effects of hormones in infants may be influenced by gender and breastfeeding and indirectly affect growth or maturation of the lungs or the immune response.⁴¹

Several potential explanations can be advanced to explain why these gender-related differences were not widely reported in the past. First, the difference in disease severity might have gone unnoticed in classic univariate analyses of gender, because unless risk analysis were simultaneously stratified by gender and lactation, differences between subgroups would not be detected. Second, gender-specific susceptibility to breast milk and severe disease may selectively reflect results in an urban population of infants at high risk in Argentina; however, reaffirming our observations, female-specific benefits from breastfeeding were described in a retrospective case-control study of term infants in Boston¹⁷ and a female-specific effect observed in wheezing infants in Arizona.¹⁸ Third, because our study recruited infants on enrollment to the clinics and not at birth, high mortality in a group of hypersusceptible boys in the NICU could have resulted in the unbalanced survival of a group of hypersusceptible girls and recruitment bias; however, a substantial difference in early survival between genders would have probably been suggested by excess enrollment of girls in our cohort, and enrollment rates for boys and girls were similar. Finally, no evidence of confounding bias was found when adjusting for a variety of epidemiologic and clinical variables; however, we cannot exclude the possibility that other, unmeasured confounders affected the results.

Although our study has limitations, including a relatively small sample size and a focus on a population at high risk for respiratory illness, it also has important strengths. First, our results were confirmed by using 2 different analytic strategies with different outcome variables. Second, we gathered data prospectively by frequent standardized clinical monitoring of all infants in the cohort; categorized breastfeeding using specific criteria; examined breastfeeding's effect on enrollment and at the episode; predefined criteria for evaluation of respiratory signs, symptoms, and disease severity; and monitored infants until resolution of symptoms.⁴² Finally, we controlled for important confounding variables. Although the

decision to rehospitalize a child can be subjective, rehospitalizations strongly correlated with the validated severity score. It is important that our findings be replicated in studies elsewhere and/or on reanalysis of other pediatric cohorts. Whether these observations affect other types of illnesses, such as gastrointestinal diseases, also deserves additional investigation.

CONCLUSIONS

We report that breast milk protection against severe acute lung disease is particularly strong for girls and describe a subgroup of infant girls who are at extreme risk for severe respiratory illness and may require special consideration. These findings have important implications for the understanding of the protective effects of breast milk and the mechanism of illness in respiratory disease.

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Abbreviations

VLBW	very low birth weight
RSV	respiratory syncytial virus
hMPV	human metapneumovirus
hPIV	human parainfluenza virus
BPD	bronchopulmonary dysplasia
LRI	lower respiratory infection

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FIGURE 1. Cumulative incidence rate of rehospitalization according to gender and breastfeeding status. Shown is the time to first event in breastfeeding and formula-fed female (♀) and male (♂) preterm infants during the first year of life.

TABLE 1
Respiratory Severity Score

Oxygen saturation

- 0 = Baseline value (no URI)
- 1 = Baseline value (URI)
- 2 = Decreased <5%
- 3 = Decreased 5–10%
- 4 = Decreased >10% or assisted ventilation

Respiratory rate

- 0 = Baseline value (no URI)
- 1 = Baseline value (URI)
- 2 = Increased 1–14/min
- 3 = Increased 15–30/min
- 4 = Increased >30/min or assisted ventilation

Retractions, wheezing, crackles

- 0 = No change (no URI)
- 1 = Minimal
- 2 = Mild
- 3 = Moderate
- 4 = Severe or assisted ventilation

Respiratory Score

- 0 = Baseline value (well)
 - 1 = URI
 - 2 = Mild LRI
 - 3 = Moderate LRI
 - 4 = Severe LRI
-

The respiratory score was modified from that of Groothuis et al.²⁰ The score is calculated by obtaining the mode of the 3 components (or the mean if no mode). Values for oxygen saturation, respiratory rate, and pulmonary findings are compared with values obtained at baseline. URI indicates upper respiratory tract infection.

TABLE 2Comparison of Epidemiologic Characteristics in VLBW Boys ($n = 64$) and Girls ($n = 55$)

Characteristic	Boys	Girls	<i>P</i>	All Infants
Gestational age, mean $\pm$ SD, wk	29.6 $\pm$ 2.2	30.0 $\pm$ 2.4	.25	29.8 $\pm$ 2.3
Infants $\leq$ 28 wk, <i>n</i> (%)	20 (31)	15 (27)	.63	35 (29)
Birth weight, mean $\pm$ SD, g	1141 $\pm$ 242	1171 $\pm$ 264	.51	1155 $\pm$ 252
Infants with birth weight <1000 g, <i>n</i> (%)	20 (31)	15 (27)	.63	35 (29)
Mechanical ventilation after birth, <i>n</i> (%)	44 (69)	37 (67)	.86	81 (68)
Duration NICU stay, mean $\pm$ SD, d	84.5 $\pm$ 50.0	76.0 $\pm$ 42.0	.31	80.5 $\pm$ 46.0
Discharge in respiratory viral season, <i>n</i> (%)	39 (61)	34 (62)	.92	73 (61)
Corrected gestational age, mean $\pm$ SD, d	51.9 $\pm$ 52.8	57.6 $\pm$ 54.6	.54	54.6 $\pm$ 53.4
BPD, <i>n</i> (%)	31 (48)	16 (29)	.03	47 (39.5)
One or both parents with asthma, <i>n</i> (%)	9 (15)	6 (12)	.64	15 (14)
Household below poverty line, <i>n</i> (%)	17 (27)	21 (40)	.16	38 (33)
Household with siblings <10 y of age, <i>n</i> (%)	39 (62)	21 (40)	.01	60 (52)
Maternal formal education $\leq$ 7 y, <i>n</i> (%)	61 (95)	52 (94)	.84	113 (95)
Smoking in the household, <i>n</i> (%)	19 (32)	26 (52)	.03	45 (41)
Mother's age, mean $\pm$ SD, y	27 $\pm$ 7	25 $\pm$ 7	.27	26 $\pm$ 7
Positive for virus, <i>n</i> (%)	22 (34)	16 (30)	.58	38 (32)
Breastfeeding at enrollment, <i>n</i> (%)	37 (58)	31 (56)	.87	68 (57)
Exclusive breastfeeding, <i>n</i> (%)	1 (2)	3 (6)	.23	4 (3)
Breastfeeding duration, mean $\pm$ SD, mo	7.2 $\pm$ 2.9	7.1 $\pm$ 2.7	.81	7.2 $\pm$ 2.7
$\leq$ 4	27	24	.87	51
>4	32	28	.87	60

TABLE 3
Risk for Infant Rehospitalization, First Episode: Association With Breastfeeding According to Gender

Gender	Breastfeeding	n/N (%)	Crude		Adjusted ^a	
			RR (95% CI)	p ^b	RR (95% CI)	p ^b
Female	Yes	2/31 (6.5)	0.13 (0.03–0.53)	.004	0.05 (0.01–0.37)	.003
	No	12/24 (50.0)	1.00		1.00	
Male	Yes	7/37 (18.9)	1.02 (0.36–2.90)	.968	2.46 (0.44–13.88)	.598
	No	5/27 (18.5)	1.00		1.00	

RR indicates relative risk; CI, confidence interval.

^a Adjusted for maternal age, household income, tobacco use at home, asthma in parents, severity of BPD, siblings <10 years of age, maternal education, gestational age, birth weight, ventilatory support, virus positive, and length of NICU stay.

^b Significance of the relative risk for infant rehospitalization, first episode (breastfeeding no/breastfeeding yes) (robust Poisson multiple regression).

^c Interaction between breastfeeding and gender.

TABLE 4
Mean Number of Episodes of Rehospitalization According to Gender and Breastfeeding Status

Gender	Breastfeeding	No. of Episodes Per Infant	Incidence Rate	Crude		Adjusted ^a	
				Ratio (95% CI) ^b	p ^b	Ratio (95% CI) ^b	p ^c
Episodes of rehospitalization	Female						
	Yes	2/31	0.065	0.05 (0.01–0.23)	.000	0.02 (0.00–0.51)	.017
	No	30/24	1.250				
Male	Yes	11/37	0.297	0.38 (0.12–1.17)	.093	0.86 (0.23–3.29)	.827
	No	21/27	0.778				
Episodes of acute respiratory infection	Female						
	Yes	89/31	2.871	0.85 (0.58–1.25)	.408	1.05 (0.67–1.65)	.824
	No	81/24	3.375				
Male	Yes	118/37	3.189	0.99 (0.68–1.44)	.957	1.03 (0.72–1.48)	.868
	No	87/27	3.222				

^a Adjusted for maternal age, household income, tobacco use at home, asthma in parents, severity of BPD, siblings <10 years of age, maternal education, gestational age, birth weight, ventilatory support, virus positive, and length of NICU stay.

^b Ratio of mean number of hospital readmissions (breastfeeding no/breastfeeding yes) (negative binomial multiple regression).

^c Interaction between breastfeeding and gender.